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ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR RISK IN PATIENTS WITH ANKYLOSING SPONDYLITIS

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Background. *Ankylosing spondylitis is a disease that induces damage to the musculoskeletal system. Mortality rate among patients with AS is in 1.5 times higher than the population level. It is caused by cardiovascular disease and chronic renal failure.*

Objective. *The research was aimed to study the prevalence of endothelial dysfunction and to establish its dependence on the factors of cardiovascular risk in patients with AS.*

Methods. *104 patients with ankylosing spondylitis (AS) were examined using standard diagnostic methods, such as disease activity, lipidogram, ultrasound of the carotid artery intima media, and endothelium vasodilatation in response to reactive hyperaemia was evaluated. Clinical activity of the disease was determined using the disease activity index BASDAI, BASFI functional index, index BASMI metrology, ASQoL quality of life. To estimate the 10-year risk of cardiovascular disease, the QRISK scale was used.*

Results. *Endothelial dysfunction (ED) was found in 47% cases. It was established that in the patients with ED<10% the incidence of LPL>1.7 mg/L, HDL-C<1.0 mmol/L, TIM thickening>0.9 mm was higher than in the patients with ED>10%. In this group of patients, significant duration of the disease and essential differences in their progress in terms of VAS, CRP, ESR, index activity and functional disorders were revealed.*

Conclusions. *The problem of CVD in patients with AS may be caused by systemic inflammatory disease associated with the development of endothelial dysfunction and increased levels of atherogenic lipids.*

KEY WORDS: **ankylosing spondylitis; endothelial dysfunction; cardiovascular risk.**

Introduction

Ankylosing spondylitis (AS) is a chronic systemic inflammatory disease primarily involving the axial skeleton (sacroiliac, intervertebral joints) [1; 12] and belongs to the group of seronegative spondylitis (SnA).

Mortality among patients with AS is in 1.5 times higher than the population levels. [8] It is caused by cardiovascular disease and chronic renal failure [3]. It is established that the presence of chronic systemic inflammation is an important predictor of cardiovascular (CV) disease [5; 7; 12] due to the development of endothelial dysfunction, and further – atherosclerosis and atherothrombosis, remodelling of vascular wall and myocardium [10] and, therefore, the main cause of numerous life-threatening adverse conditions.

Specifics of endothelial dysfunction development have been well studied in patients with rheumatoid arthritis, systemic lupus erythematosus. At the same time, despite widespread prevalence of AS, this aspect has not been discussed and analyzed in a cohort of these patients so far. There is scanty information of the impact of systemic inflammation activity on the functional state of endothelium in cases of AS, and of the possibility of endothelial dysfunction correction against the background of anti-inflammatory therapy. The research was aimed to study the prevalence of endothelial dysfunction and to establish its dependence on the factors of cardiovascular risk in patients with AS.

Methods

104 patients (90 males and 14 females) with verified diagnosis of AS were examined. They were hospitalized into the Department of Rheumatology of Ternopil University Hospital within 2015-2017. The study inclusion criteria were: the diagnosis of AS according to the modified New York criteria and the informative patient's consent to participate in the study. Exclusion criteria were age over 60 years old, presence of psoriasis, Crohn's disease, ulcerative colitis, coronary heart disease, manifes-

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tations of peripheral atherosclerosis, clinically significant heart disease, circulatory insufficiency of any origin, diabetes, severe liver disease, kidney diseases and other chronic diseases in their acute phases. All patients who agreed to participate in the study underwent general clinical examination (common blood test, urinalysis, ECG, X-ray of sacroiliac joints), biochemical test of blood with determination of lipidograma and acute phase indicators (C-reactive protein, rheumatoid factor and etc.) and detection of HLA B-27. Clinical activity of the disease was determined using the disease activity index BASDAI, BASFI functional index and BASMI metrology index, ASQoL quality of life [2, 4]. Endothelium vasodilatation was evaluated in response to reactive hyperaemia in 57 patients by the method, which was first described by D. Celermajer [9], using Acuson 128 XP/10 ultrasonic complex equipped with a 7 MHz linear transducer. The study was conducted in a duplex mode (ultrasound scanning in B-mode and Doppler spectral analysis of the signal). Tests with reactive hyperaemia (*endothelium-dependent vasodilators*) and nitro-glycerine (*endothelium-independent vasodilators*) were performed [10].

Endothelium-dependent vasodilatation (EDVD) was calculated by the formula: EDVD=(d60–d0)×100%/d0, where d60 is the brachial artery diameter 60 seconds after the restoration of blood flow, d0 – the initial diameter of brachial artery. *Endothelium-independent* vasodilatation (EIVD) was calculated by the formula: EIVD=(d5–d0)×100%/d0, where d5 – the diameter of brachial artery after 5 minutes of taking nitro-glycerine, d0 – the initial diameter of brachial artery.

To determine the correlation between EIVD and EDVD, the reactivity index (IR) of brachial artery was calculated using the formula: IR=EIVD/EDVD. To assess the 10-year risk of cardiovascular disease, the scale QRISK was used (Q-RESEARCH Cardiovascular Risk Algorithm). The advantage of this scale is the use of such risk factors: age, sex, smoking, systolic blood pressure, the ratio of total cholesterol and HDL cholesterol, body mass index, family history of coronary artery disease, socioeconomic status, treatment with antihypertensive drugs and the presence of comorbidity, systemic inflammatory disease in an individual case.

Statistical analysis of the results was carried out using traditional methods of ANOVA, SPSS 22 (© SPSS Inc.).

Results

The main part of patients was of working age (mean age – 35.9±17.1 years old, duration of the disease averaged 15.9±8.2 years (Fig. 1)). Peripheral form of the disease was found in 35 patients (33.7%), central form – in 69 patients (66.3%). Evaluation of the disease proved the presence of high and moderate activity in the absence of disease in patients who had inactive or active course.

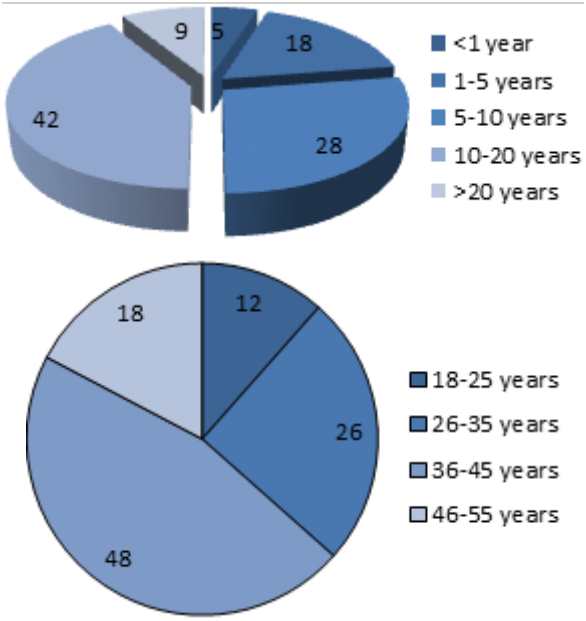

Fig. 1. Classification of patients according to age and disease duration

The research of endothelium functions in the patients with AS and in the control group proved that remodulation of vessels occurred due to endothelium dilation of brachial artery in response to reactive hyperaemia and that they differ slightly in the quantitative value of the indicator in the control group. Besides, EDVD reduced (less than 10%), that is a sign of endothelial dysfunction, more common among patients with AS as compared to the control group (45.7% vs. 11.8%, respectively). EIVD is significantly higher than EDVD in patients with AS and healthy individuals. However, EIVD in the patients with AS was found to exceed significantly the EDVD in the control group (Table 1).

In what follows, we evaluated clinical and laboratory characteristics of AS in patients with reduced EIVD (Table 2). The data proved that in the patients with impaired endothelial vasoregulation function, a significantly longer duration of disease was revealed, the onset of the illness symptoms was evidenced in younger

Table 1. Results of endothelia vasoregulation function

Indices	AS (n=57)	The control group (n=20)	p
The initial diameter of brachial artery, mm	4.32±0.29	3.78±0.18	p<0.05
The wall thickness of brachial artery, mm	0.58±0.06	0.41±0.03	p<0.05
The output speed of blood flow, m/s	0.72±0.09	0.68±0.07	p<0.05
EDVD, %	9.8±1.23	13.3±1.1	p<0.05
EIVD, %	25.4±2.14	19.7±5.4	p<0.05
Brachial artery reactivity index	2.35±0.12	1.37±0.14	p<0.05

Note: p – significant differences between the baselines indicators of the study and control groups.

Table 2. Clinical and laboratory characteristics of patients with AS

Indices	EDVD>10% (n=31)		EDVD<10% (n=26)		p
Disease duration, (yrs)	13.7±5.2		16.1±4.8		p>0.05
Age of disease onset, (yrs)	25.8±3.2		23.2±2.1		p>0.05
The presence of peripheral arthritis	n	%	n	%	p>0.05
	10	32.3	9	34.6	
BASDAI, sm	4.64±0.42		6.80±0.27		p<0.05
BASFI, sm	4.34±0.28		5.74±0.18		p<0.05
DFI, points	15.8±1.1		17.1±0.89		p<0.05
VAS, mm	61.9±14.5		80.3±12.4		p<0.05
C-reactive, mg/l	8.3±1.4		15.2±2.7		p<0.05
ESR, mm/h	18.3±3.2		28.5±4.1		p<0.05

Note: p – significant differences between ED<10% and ED>10%.

age. In addition, the patients with impaired EDVD had significantly higher indices of VAS and activity (BASDAI) with significantly higher than quantitative indicators of CRP, ESR, functional disorders and DFI.

Among the patients with EIVD<10% (26 patients), 14 (53.8%) are treated with NSAIDs, only 4 (28.5%) of them – continuously. Among the patients with EDVD>10% (31 patients), 28 persons underwent NSAIDS therapy, 19 of them – continuously (Fig. 2)

Classical cardiovascular risk factors and arterial hypertension are crucial for the development of endothelial dysfunction, so their prevalence in the group of the examined patients was analysed (Table 3).

About every third patient suffered from II degree hypertension, and the frequency of its registration was found to be similar to the prevalence among the general population (in the city area – 29.3%, in rural – the prevalence of hypertension – 36.3%) [13].

It should be noted that adequate antihypertensive therapy at the time of inclusion into the study was provided for 14 patients (39%). Thickening of intima-media common carotid artery >1 mm was found in 19.2% patients with AS. High incidence of smoking among patients with AS can be due to the predominance of males. Many patients had reduced levels of HDL and high LDL levels that can be attributed to scanty availability of patients with hypercholesterolemia.

Mean BMI in the studied patients was 20.2±4.9 kg/m^2; 72 (69.2%) patients had normal weight (BMI 18.5-24.9 kg/m^2), 18 (17.3%) patients had a deficit of body weight (BMI<18.5 kg/m^2),

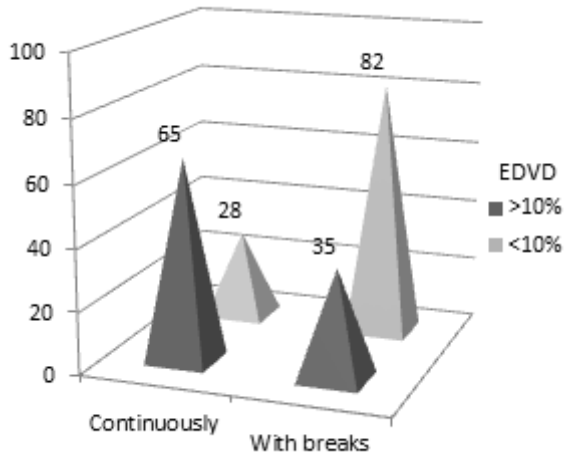

Fig. 2. Dependence of EDVD on the duration of NSAID treatment

Table 3. The prevalence of traditional cardiovascular risk factors

Sign	n	%
Arterial hypertension	23	22.1
TIM of common carotid artery >1 mm	49	47.1
Smoking	34	32.7
Hypercholesterolemia (TCh>5.2 mmol/l)	22	21.2
Low level of HDL (<1 mmol/l for male and <1.2 mmol/l for female)	63	60.6
High level of LDL (>3.0 mmol/l)	41	39.4
Hypertriglyceridemia (TГ >1.7 mmol/l)	14	13.5
Overweight (BMI>25 kg/m^2)	12	11.5
Family history of early heart disease	16	15.4

and 10 (9.6%) patients had excessive body weight (BMI>25 kg/m^2), including 4 (3.8%) with obesity (BMI>30 kg/m^2).

The next stage of our research was aimed at analysing the quantitative characteristics of cardiovascular risk factors in patients with impaired EIVD (Table 4). The data proved that the level of atherogenic lipids (LDL and HDL) was the sole indicator of significant differences between patients with normal and impaired EIVD. On the other hand, it was found that patients in groups with normal and low EDVD were comparable regarding most cardiovascular risk factors. Thus, traditional factors are unlikely to play a major role in the processes of endothelial wall remodullation and EDVD occurrence.

Further cardiovascular risk for the examined patients was established, the value for the study group (3.4 % [1.8; 5.4]) being about in three times higher than the risk value for the general population. It should be noted that the average cardiovascular risk in the patients with impaired EDVD was 2.8% as compared to the index in normal EIVD – 3.9%.

Discussion

Brachial artery active response to exogenous nitrate in patients with AS proves more comparable index of reactivity of brachial artery, that is indicative of the ratio between EIVD and EDVD. A recent research suggests that excessive response of brachial artery to exogenous nitrate in patients with AS is associated with deficient endogenous production of nitric oxide and can be regarded as a sign of endothelial dysfunction [5, 6]. The obtained findings prove

Table 4. Summary of cardiovascular risk factors and hypertension in patients with normal and reduced endothelium vasodilation

Markers	EDVD >10% (n=31)		EDVD <10% (n=26)		p
Average age, years	36.8±8.2		38.8±8.8		p>0.05
BMI, kg/m	22.8±4.3		23.9±4.1		p>0.05
Smoking	n	%	n	%	
	12	39	10	38	p>0.05
History of smoking, years	10.2±2.4		9.2±1.9		p>0.05
Family history of early heart disease	n	%	n	%	
	6	19	4	15	p>0.05
TCh, mmol/l	4.52±0.94		4.28±0.81		p>0.05
HDL, mmol/l	1.16±0.31		0.71±0.21		p<0.05
LDL, mmol/l	2.61±0.18		3.22±0.29		p<0.05
Tg, mmol/l	1.15±0.44		1.32±0.58		p>0.05
Atherogenic index	3.4±1.21		3.9±1.26		p>0.05
TIM of common carotid artery, mm	0.89±0.12		1.32±0.18		p<0.05
Arterial hypertension	n	%	n	%	
	7	22.5	6	23.1	p>0.05
Systolic arterial pressure, mm.hg.	124.5±18.3		122.5±22.5		p>0.05
Diastolic arterial pressure, mm.hg.	78.5±14.5		76.5±18.5		p>0.05

Note: p – significant differences between ED<10% and ED>10%.

that output diameter and wall thickness of brachial artery in the patients with AS were significantly higher than similar indices in the control group, and the rate of blood flow through the artery was virtually identical. This may evidence the signs of brachial artery remodelling in patients with AS, endothelium is crucial [5, 6].

The detected changes of lipid profile are typical for the patients with systemic inflammatory diseases, and are consistent with the literature [11].

It should be emphasized that a high cardiovascular risk in patients with AS cannot be explained from the standpoint of the analysis of classical risk factors, as they do not explain high incidence of patients with endothelium dependent vasodilatation, which is a significant predictor of cardiovascular problems in patients with inflammatory diseases of joints.

Conclusions

Our findings suggest high prevalence of ED in patients with AS (47% cases). ED <10% caused significant increase in the number of patients with LDL >1.7 mmol/dL, HDL-C <1.0 mmol/L, TIM thickening >0.9 mm. At the same time, in this group of patients a significantly longer duration of disease and substantial differences in the course in VAS, CRP and ESR indices, as well as activity index and functional disorders were revealed. With the QRisk score scale, SS risk was established as 3.4% that is about three times higher than the risk among the general population. Among the traditional factors, increased HDL and decreased LDL are most often reported. Thus, the problem of CVD is one of the most important aftermaths of systemic inflammatory disease that is associated with the development of endothelial dysfunction and increased levels of atherogenic lipids.

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SERUM SOLUBLE CD25 IN HEPATOCELLULAR CARCINOMA, SHALL WE BE ABLE TO CHANGE THE NATURAL HISTORY?

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Background. *Although hepatocellular carcinoma (HCC) is one of the most common malignancy related mortality worldwide, it can be curable if detected in early stages. Emergence of a new marker that can early detect HCC could help in early treatment and therefore ameliorate the outcome.*

Objective. *The aim of the research is to evaluate the performance of serum soluble CD25 (sCD25) in the prediction of early HCC and compare it to α-fetoprotein (AFP).*

Methods. *Serum levels of sCD25 and AFP were measured in three groups of population; HCC group (40 patients), cirrhosis without HCC control group (20 patients) and healthy control group (20 patients). HCC group contained 20 early and 20 late stage patients according to Barcelona Clinic Liver Cancer (BCLC) staging system (stage 0/A and B-D respectively). Levels of both biomarkers were compared in all groups. Predictive yield of both biomarkers for early HCC was evaluated using ROC curve analysis.*

Results. *Level of sCD25 was significantly higher in patients with HCC than in both cirrhotic controls and healthy controls (P<0.0001and 0.013 respectively). For prediction of early HCC in patients with cirrhosis, the optimal sCD25 cut-off level was 7.15 ng/ml with sensitivity and specificity of 90% and 60% respectively (AUC=0.717; P=0.019) while sensitivity and specificity of AFP were 70% and 85% respectively at a cut-off value of 9.85 ng/ml (AUC=0.781; P=0.002) in the same settings.*

Conclusion. *sCD25 seems to be a reliable biomarker for early detection of HCC and therefore could enhance the outcome.*

KEY WORDS: **hepatocellular carcinoma; soluble CD25; alfa fetoprotein.**

INTERNAL MEDICINE

Introduction

Hepatocellular carcinoma is one of the most serious and life threatening complications of chronic liver disease. It represents the 5th most common malignancy in men, the 7th in women and the 3rd malignancy related mortality worldwide. Curative treatment strategy can be achieved if detected in early stages [1–4]. The role of serum α-fetoprotein (AFP), the widely used classical biomarker for HCC, has been stepped down in the recent European and American surveillance guidelines because of low sensitivity and specificity. This is based on the knowledge that almost 80% of small HCCs do not show increased levels of AFP, and the sensitivity decreases to 25% in tumors smaller than 3 cm [5–8]. Looking for a new marker with a better diagnostic accuracy became an inevitable requirement. This eventually would optimize the HCC surveillance program and improve the outcome through prompt application of the proper treatment strategy early in the course of the disease. Serum soluble CD25 (sCD25) has been recently investigated as a new marker for hepatocellular carcinoma. It quantitatively reflects the immunological activity against the tumor [9–11]. It represents the α-chain of interleukin 2 receptor (IL-2Rα) which is composed of three polypeptide chains: α, β and γ. It is not found on the surface of resting T cells, but rapidly expressed on their surface after being activated. Chronic T-cell stimulation, as in some malignancies, leads to shedding of IL-2Rα (CD25) into plasma with subsequent elevation of its level [11–16]. Cabrena and colleagues reported that serum level of sCD25 was correlating with tumor burden and poor survival in HCC patients and believed that measuring

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serum level of sCD25 might provide a clue for early diagnosis of HCC [12]. When we designed the current study, we hypothesized that sCD25 could have an impressive diagnostic value and a potential ability for detection of early HCC. We assessed the performance of sCD25 in the prediction of early HCC and its correlation with the tumor stage and compare it with AFP.

Methods

The study was conducted in National Liver Institute, Menoufiya, Egypt. After obtaining an informed consent, eighty persons in 3 groups were included; HCC on a background of cirrhosis (40 patients), liver cirrhosis with no evidence of HCC (20 patients) and healthy control group (20 patients). HCC group comprised 20 early and 20 late stage HCC patients, according to Barcelona Clinic Liver Cancer (BCLC) staging system, (stage A and B-D respectively) (Fig. 1). Cirrhotic and healthy controls had matched age and sex with HCC patients. All included cases of HCC was diagnosed on the basis of the presence of typical vascular enhancement pattern of liver lesion (s) in contrast enhanced dynamic CT scan or MRI [18]. Diagnosis of cirrhosis was based on combined historical, clinical, laboratory and radiological findings. Severity of cirrhosis was assessed by Child Pugh classification [19]. All patients had complete laboratory profile including CBC, liver panel, creatinin as well as serum level of sCD25 and AFP. ELISA kit (Elecsys E411, Switzerland) was used to quantify blood level of AFP while ELISA kit (Bender MedSystems, Vienna, Austaria) was used to measure serum level of sCD25.

Statistical methods

SPSS, version 21 for Windows (Inc, Chicago, IL, USA) was used for all statistical analyses. Qualitative data were presented as frequency and percentage. Chi square and Fisher's exact tests were used to compare groups. Quantitative data were presented as mean and standard deviation. For non-parametric data, Student t-test and Mann–Whitney U test were used to compare level difference of sCD25 between two groups while ANOVA and Kruskal Wallis were used to compare level difference of sCD25 between more than two groups. Receiver-operator characteristic (ROC) curve analysis was used to generate sensitivity and specificity at different cut-offs. The best cut-off was set at the value where sensitivity and specificity were maximal. Correlation between serum level of sCD25 and laboratory parameters was assessed by Spearman's correlation coefficient. The statistical significance was set at P-value of less than 0.05 for all tests.

Results

The studied populations were mostly males representing 77.5, 75 and 60% in HCC, cirrhotic and healthy control groups respectively. The mean age was 56.38±5.934 years in HCC group while was 53.75±7.383 and 54.20±5.863 years in cirrhotic and healthy controls respectively. Hepatitis c virus (HCV) was the underlying etiology of cirrhosis in all patients in both HCC and cirrhotic control groups. The mean sCD25 level was 13.07±6.645, 13.15±6.967, 8.938±6.487 and 4.97±3.031 ng/ml in early HCC, late HCC, cirrhotic and healthy control groups respectively. Level of sCD25 was significantly higher in patients with HCC than in both cirrhotic and healthy controls ($p<0.0001$ and 0.013 respectively) and significantly higher in cirrhotic patients than healthy controls ($p=0.042$). sCD25 level was significantly and positively correlated with the severity of liver disease as assessed by Child-Pugh classification ($r=0.56$, $p<0.001$). There was no statistical difference between sCD25 in early and late HCC ($p=0.968$). The mean AFP level was 17.66±12.092, 244±302.041, 8.01±6.965 and 2.95±2.175 ng/ml in early HCC,

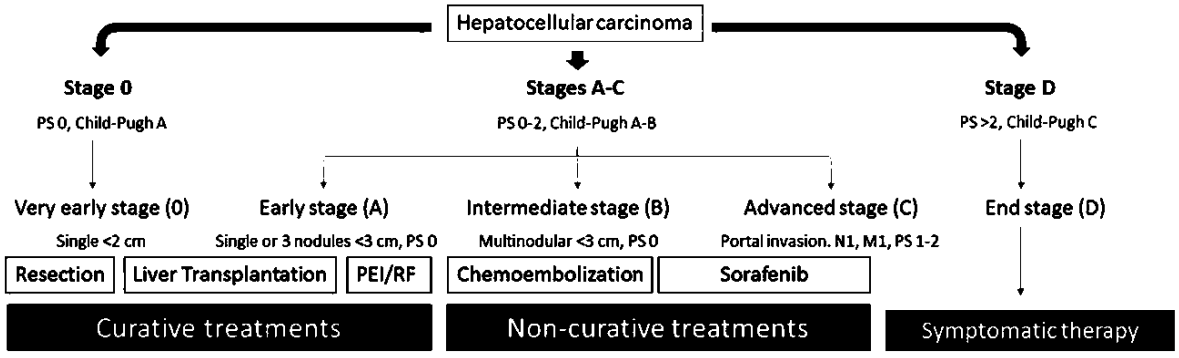

Fig. 1. Barcelona Clinic Liver Cancer (BCLC) staging and treatment strategy (Adapted from Llovet JM, et al. Lancet 2003) [17]. PS – performance status; PEI – percutaneous ethanol injection; RF – radiofrequency

Table 1. Statistical difference of demographic and laboratory data among the studied groups

		Total HCC (n=40)	Early HCC (n=20)	Late HCC (n=20)	LC (n=20)	Healthy control (n=20)	*p*	*p**	*p^*	*p#*
Sex n (%)	♂s	31 (77.5)	15 (75)	16 (80)	15 (75)	12 (60)	0.156	0.311	0.829	0.705
	♀s	9 (22.5)	5 (25)	4 (20)	5 (25)	8 (40)				
Age (years)		Mean±SD					0.212	0.822	0.133	0.539
		56.38± 5.934	58.40± 5.576	55.35± 5.706	53.75± 7.383	54.20± 5.863				
Hb (g/dl)		11.07± 1.097	11.14± 1.268	11.01± 0.925	10.52± 0.928	12.71± 1.091	<0.001	<0.001	0.058	0.724
WBCs (×103/dl)		4.88± 1.717	5.18± 2.247	4.59± 0.903	4.87± 1.242	7.00± 1.693	<0.001	<0.001	0.977	0.282
Platelets (×103/dl)		119.65± 35.246	122.55± 34.264	116.75± 36.854	169.05± 31.749	217.80± 47.522	<0.001	<0.001	<0.001	0.609
INR		1.37± 0.196	1.43± 0.197	1.32± 0.185	1.31± 0.236	1.07± 0.081	<0.001	<0.001	0.225	0.091
Albumin (g/dl)		3.19± 0.371	3.334± 0.382	3.04± 0.299	3.55± 0.445	4.34± 0.463	<0.001	<0.001	0.002	0.009
Bilirubin (mg/dl)		1.64± 0.833	1.19± 0.415	2.09± 0.907	1.73± 0.692	0.84± 0.154	<0.001	<0.001	0.626	<0.001
ALT (U/ml)		65.15± 15.184	61.75± 17.278	68.55± 12.262	57.05± 10.655	24.45± 5.276	<0.001	<0.001	<0.001	0.159
AST (U/ml)		89.48± 24.724	76.50± 17.021	102.45± 24.708	67.85± 10.069	27.25± 4.962	<0.001	<0.001	0.019	<0.001
Creatinin (mg/dl)		0.93± 0.159	0.93± 0.180	0.94± 0.139	0.95± 0.161	1.04± 0.193	0.025	0.114	0.665	0.845
sCD25 (ng/ml)		13.11± 6.719	13.07± 6.645	13.15± 6.967	8.938± 6.487	4.97± 3.031	<0.001	0.042	0.013	0.968
AFP (ng/ml)		130.83± 240.106	17.66± 12.092	244± 302.041	8.01± 6.965	2.95± 2.175	0.008	0.926	0.010	0.003
Child-Pugh score n (%)	A	6 (15)	6 (30)	0 (0)	12 (60)	NA	NA	NA	0.001	0.004
	B	29 (72.5)	14 (70)	15 (75)	8 (40)					
	C	5 (12.5)	0 (0)	5 (25)	0 (0)					

AFP – α-fetoprotein; Hb – hemoglobin; HCC – hepatocellular carcinoma; INR – international normalized ratio; LC – liver cirrhosis; NA – not applicable; *p* – significance between HCC and healthy controls; *p** – significance between liver cirrhosis and healthy controls; *p^* – significance between HCC and liver cirrhosis; *p#* – significance between early and late HCC; sCD25 – soluble CD25; ♂s – males; ♀s – females.

Table 2. Correlation between sCD25 and laboratory parameters among the studied groups

	Total HCC (n=40)		Early HCC (n=20)		Late HCC (n=20)		LC (n=20)		Control (n=20)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Hb (g/dl)	-0.060	0.714	-0.038	0.875	0.040	0.866	0.304	0.193	-0.371-	0.118
WBCs (×10³/dl)	-0.228	0.157	-0.478	0.033	-0.063	0.792	-0.081	0.736	0.179	0.462
Platelets (×10³/dl)	0.128	0.431	0.068	0.777	0.290	0.215	-0.136	0.567	-0.269	0.265
INR	0.151	0.352	0.250	0.287	0.039	0.869	-0.224	0.343	0.035	0.887
Albumin (g/dl)	0.002	0.991	0.205	0.387	-0.220	0.352	0.142	0.550	0.064	0.794
Bilirubin (mg/dl)	-0.038	0.816	-0.102	0.668	-0.021	0.928	-0.442	0.051	0.266	0.270
ALT (U/ml)	0.093	0.570	0.078	0.745	0.049	0.838	-0.014	0.955	0.348	0.144
AST (U/ml)	0.124	0.445	0.179	0.450	0.078	0.744	-0.078	0.744	0.390	0.099
Creatinin (mg / dl)	0.062	0.706	0.136	0.569	-0.043	0.856	-0.217	0.359	-0.249	0.303
AFP (ng/ml)	0.023	0.890	0.196	0.407	-0.093	0.697	-0.254	0.279	0.503	0.028

AFP – α-fetoprotein; Hb – hemoglobin; HCC – hepatocellular carcinoma; INR – international normalized ratio; LC – liver cirrhosis; r – Spearman's correlation coefficient.

late HCC, cirrhotic and healthy control groups respectively with statistical difference between HCC versus cirrhotics and early versus late HCC as well (p=0.010 and 0.003 respectively). The rest of demographic and laboratory data as well as their statistical differences between the studied groups are presented in Table 1. Correlation analyses between sCD25 and laboratory parameters among the studied groups are presented in Table 2. There was no significant correlation with all laboratory parameters apart from a negative correlation with WBCs in early HCC group (r=-0.478, p=0.033) and a positive correlation with AFP in healthy control group (r=-0.503, p=0.028). sCD25 performed well in predicting HCC presence among patients with cirrhosis; sensitivity and specificity were 90% and 84.2% respectively at a cut-off value of 7 ng/ml (AUC=0.969; p<0.0001). For prediction of early HCC in patients with cirrhosis, the optimal sCD25 cutoff level was 7.15 ng/ml with sensitivity and specificity of 90% and 60% respectively (AUC=0.717; p=0.019) while, sensitivity and specificity of AFP were 70% and 85% respectively at a cut-off value of 9.85 ng/ml (AUC=0.781; p=0.002) in the same settings (Fig. 2).

Discussion

HCC represents the most serious and lethal complication of cirrhosis. Fortunately, early stages of HCC could be curative. Axiomatically, detection of HCC in early stages would be helpful in changing the poor outcome of late stages by offering the proper treatment early in the course of the disease with subsequent amelioration of the outcome [20–22]. In the current study, we evaluated the performance of sCD25 in predicting early HCC stages among patients with cirrhosis and compare it to AFP. Serum sCD25 level was significantly higher in HCC patients than cirrhotics (p<0.0001) and healthy controls (p=0.013). In the same stream, it was significantly higher in cirrhosis than healthy controls (p=0.042). Additionally, there was a significant positive correlation between serum sCD25 and severity of cirrhosis (Child-Pugh class) (r=0.56, p<0.001). The optimal sCD25 cut-off level in detecting early HCC among cirrhotic patients was 7.15 ng/ml with sensitivity and specificity of 90% and 60% respectively (AUC=0.717; p=0.019). On the other hand, sensitivity and specificity of AFP were 70% and 85% respectively at a cut-off value of 9.85 ng/ml (AUC=0.781; p=0.002) in the same settings .This higher sensitivity of sCD25 highlights its substantial role as a screening marker for HCC. Similar findings were reported by Cabrena and his group. They reported sCD25 cut-off level of 2899 pg/ml as the best cut-off with a sensitivity of 89.6% and a specificity of 39.3% (AUC=0.630, p<0.0001). By comparison,

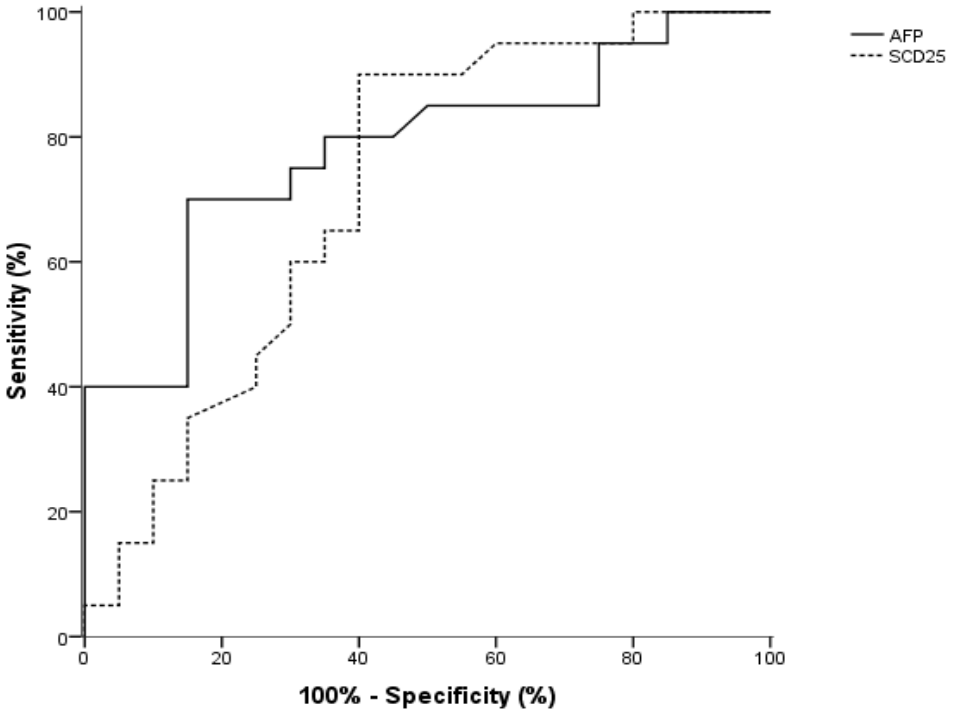

Fig. 2. Receiver operator curve (ROC) of sCD25 and AFP levels for the prediction of early HCC among patients with cirrhosis

at a cut-off value of 20 ng/ml, AFP had a sensitivity of 41.7% and a specificity of 82.6% (AUC=0.630, p=0.0257) [12] The difference between the optimal cuto-ff between the current study (7150 pg/ml) and that of Cabrena et al. (2899 pg/ml) might be referred to the variability in the sample size, underlying etiology as well as dissimilarity in racial, ethnic, genetic and environmental factors. It is noteworthy that, the main underlying etiology of liver disease was HCV representing 92.5 and 90% in HCC and cirrhosis groups respectively while 7.5 and 10% were referred to combined HCV and HBV etiology in the same groups respectively. In the study of Cabrena et al., 60% were HCV, 13% were cryptogenic, 9% were alcoholic cirrhosis and 9% were non-alcoholic fatty liver disease (NAFLD) in HCC group while 72% were HCV, 5% alcoholic cirrhosis and 5% NAFLD and 3% were cryptogenic in cirrhosis group. In spite of the presence of a significant positive correlation between serum levels of sCD25 and severity of liver cirrhosis, there was no significant difference in its level in early and late HCC which disclaims findings of Cabrena et al., who reported a significant positive correlation between serum levels of sCD25 and tumor stage [12]. We could not eventually find a reasonable explanation for these conflicting results however difference in underlying etiology, tumor differentiation/biology, inter-racial and inter-ethnic variations between both studies might be accused. A notable finding that should be considered the correlation between sCD25 and AFP in HCC and cirrhosis groups was absent denoting that measuring both markers in serum can improve the reciprocally holistic diagnostic value of HCC.

Conclusions

Serum sCD25 sounds to be a good marker for predicting early HCC. There was some discrepancy between the optimal cut-off in the current and previous studies. This calls for a large scale study for further integration and unification of the current results and previous ones and to standardize the optimal cut-off taking into consideration addressing the relationship between sCD25 level and tumor biology rather than tumor size and number.

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LEFT VENTRICULAR DIASTOLIC DYSFUNCTION AND OXYGEN SUPPLY OF LOWER EXTREMITIES IN PATIENTS WITH STABLE ISCHEMIC HEART DISEASE AND CONCOMITANT TYPE 2 DIABETES MELLITUS

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Background. *The peculiarities of diastolic heart failure and indices of arterial and venous blood oxygenation in patients with stable ischemic heart disease and concomitant type 2 diabetes mellitus are presented in the article. Obvious left ventricular diastolic dysfunction with the increased levels of natriuretic peptide, uric acid and decreased indices of arterial and venous blood oxygenation in the presence of comorbid type 2 diabetes mellitus have been revealed.*

Objective. *The research was aimed to study the peculiarities of left ventricular diastolic function disorders, levels of NT-proBNP, uric acid and indices of arterial and venous blood oxygenation in patients suffering from stable exertional angina with underlying comorbid type 2 diabetes mellitus.*

Methods. *70 patients with IHD: stable exertional angina of the III functional class, were examined. The first group comprised 39 patients with stable exertional angina of the III functional class with left ventricular diastolic dysfunction; the second group – 31 patients with stable exertional angina of the III functional class with left ventricular diastolic dysfunction and concomitant type 2 diabetes mellitus. All the examined patients underwent BD- echocardioscopy, with the detailed evaluation of left ventricular diastolic function, NT-proBNP and uric acid levels in venous blood were determined by immunoenzyme method, indices of arterial and venous blood oxygenation were evaluated too.*

Results. *The correlation between left ventricle diastolic function and oxygen volume consumed by the tissues of lower extremities in patients with stable ischemic heart disease and concomitant type 2 diabetes mellitus was determined.*

Conclusions. *In patients with stable IHD, left ventricular diastolic dysfunction and concomitant type 2 diabetes mellitus the levels of NT-proBNP, uric acid and oxygen supply of lower extremities are significantly higher as compared to patients with IHD without type 2 diabetes mellitus.*

KEY WORDS: **stable ischemic heart disease; left ventricular diastolic dysfunction; diabetes mellitus; natriuretic peptide; blood oxygenation.**

Introduction

Stable ischemic heart disease (IHD) in patients with type 2 diabetes mellitus is diagnosed in 2–4 times more often than in people of the same age without diabetes [1]. According to some authors, diabetes mellitus negatively influences on left ventricular diastolic function, and the increase of diabetes mellitus duration is accompanied by chronic heart failure development [4]. There are some findings that left ventricular diastolic dysfunction may be caused by diabetes mellitus development regardless of the presence of IHD or arterial hypertension [2]. The results of some researches show that the prevalence of asymptomatic left ventricular diastolic dysfunction in patients with type 2 diabetes mellitus is 63.2 % and increases with age [4]. One of the main markers of chronic heart failure is N-terminal brain natriuretic propeptide (NT-proBNP), its levels increase as chronic heart failure develops [3]. An asymptomatic hyperuricemia is also an important risk factor in the development of cardiovascular complications. Therefore early diagnostic of this pathology is important for proper drug-induced correction in patients with IHD, left ventricular diastolic dysfunction and concomitant type 2 diabetes mellitus [5].

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The research was aimed to study the peculiarities of left ventricular diastolic dysfunction, the levels of NT-proBNP, uric acid and indices of arterial and venous blood oxygenation in patients with stable exertional angina with comorbid type 2 diabetes mellitus.

Methods

70 patients with IHD: stable exertional angina of the III functional class, were examined. The average age of the examined patients was (58.38±0.64) years old. The first group comprised 39 patients suffering from stable exertional angina of the III functional class with left ventricular diastolic dysfunction; the second group counted in 31 patients with stable exertional angina of the III functional class with left ventricular diastolic dysfunction and concomitant type 2 diabetes mellitus. All patients were diagnosed with relaxation type of left ventricular diastolic dysfunction, and left ventricular ejection fraction in all examined patients was higher than 45%, which means that systolic function of the left ventricle was preserved. The control group comprised 20 healthy individuals of the same age and sex. All the examined patients underwent BD-echocardioscopy with the detailed evaluation of left ventricular diastolic function, NT-proBNP and uric acid levels in venous blood were determined by immunoenzyme method, indices of arterial and venous blood oxygenation were evaluated too (saturation of arterial ($Sa.O_2$) and venous ($Sv.O_2$)) blood – with pulse oximeter; arterial ($Ca.O_2$) and venous ($Cv.O_2$) blood oxygenation in vitro including the assessment of oxygen volume consumed by the tissues of lower extremities $Da.O_2$-$Dv.O_2$) – with Oximeter Unistat apparatus (USA).

All statistical analyses were performed with Statistica 6.0 and Microsoft Excel. Data are expressed as means±standard deviation or as number (%). Continuous variables were analyzed by the Student's t-test and the Mann–Whitney test. P<0.05 was considered statistically significant. The authors had full access to information and take full responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written. Institutional review committee approval and informed consents were obtained.

Results

In patients with IHD and concomitant type 2 diabetes mellitus correlation of E/A was by 34.8% (p<0.01) lower than in the control group and by 15.1% (p<0.05) higher than in patients with IHD without concomitant type 2 diabetes mellitus (Table 1). IVRT and DT values also differed considerably in patients with IHD, left ventricular diastolic dysfunction and concomitant type 2 diabetes mellitus, specifically: DT value was by 30.5% (p<0.01) higher than in the control group and by 24.1% (p<0.05) higher than in patients with IHD without type 2 diabetes mellitus. E' value was by 50.5% (p<0.01) lower in the 2nd group of patients as compared to the control group, and by 26.8% (p<0.05) lower than in patients with IHD without type 2 diabetes mellitus. In patients with stable IHD correlation of E/E' was increasing to (9.83±0.14) because of significant decrease in E' and was in 1.8 times higher than in the control group and by 25.9% (p<0.01) higher as compared to the patients with IHD without type 2 diabetes mellitus that proves an increased inflexibility of myocardium in patients with comorbidity of IHD and type 2 diabetes mellitus.

NT-proBNP value in patients with stable IHD and concomitant type 2 diabetes mellitus was (566.07±22.01) pg/ml and was by 35.6% (p<0.01) higher as compared to the first group of the examined patients and by 78.7% (p<0.01) higher as compared to the control group that evidences more significant chronic heart failure development in patients with stable IHD and concomitant type 2 diabetes mellitus (Fig. 1).

In patients with IHD, left ventricular diastolic dysfunction and concomitant type 2 diabetes mellitus the level of uric acid was by 53.3% (p<0.01) higher as compared to the control group and by 21.3% (p<0.05) higher as compared to the examined patients without con-

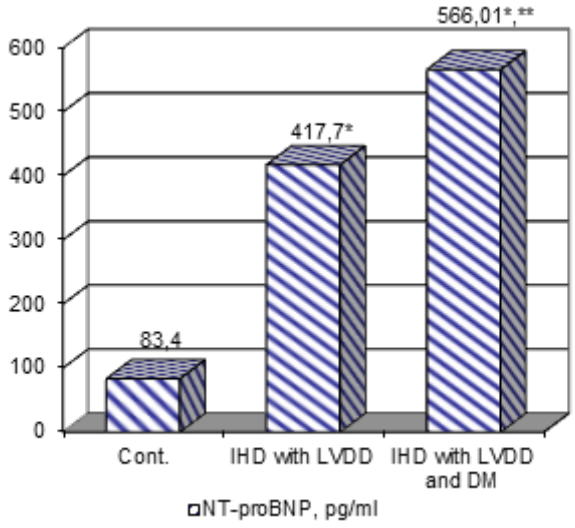

Fig. 1. Level of NT-proBNP in the examined patients

comitant type 2 diabetes mellitus. The data obtained in our research confirm the results of some authors that hyperuricemia is an independent risk factor in the development of cardiovascular complications in patients with IHD and has negative effect on the clinical course of chronic heart failure.

In patients with stable exertional angina and concomitant type 2 diabetes mellitus the decrease in arterial and venous blood oxygenation indices as compared to the patients with stable IHD without type 2 diabetes mellitus was determined (Table 2). $Sa.O_2$ was slightly lower in the 2nd group than in the 1st group. $Sv.O_2$ value in the 2 group of patients with comorbid type 2 diabetes mellitus was by 21.8% ($p<0.05$) lower as compared to the patients of the 1st group. $Ca.O_2$ and $Cv.O_2$ values were considerably lower in patients with left ventricular diastolic dysfunction and type 2 diabetes mellitus as compared to the patients without diabetes mellitus, respectively by 21.2% ($p<0.01$) and 18.5% ($p<0.01$), which indicates the deficiency of oxygen saturation of lower extremities tissues as the result of transvenous and transcapillary blood flow difficulties.

The volume of oxygen consumed by tissues of lower extremities $Da.O_2$–$Dv.O_2$ in patients with stable IHD and type 2 diabetes mellitus was (44.85±0.94) ml·l^{-1} and was by 9.6% ($p<0.05$) lower as compared to the patients without type 2 diabetes mellitus. So, in patients with stable IHD and concomitant type 2 diabetes mellitus the decrease in oxygen volume, which is transported to the tissues of lower extremities, was accompanied by the decrease in oxygen volume consumed by the tissues of lower extremities.

The direct correlation between the decrease in value of E' decline and decrease oxygen volume consumed by the tissues of lower extremities in patients with stable ischemic heart disease and concomitant type 2 diabetes mellitus was revealed.

Discussion

According to the recent literature [6,8], violation of oxygen transporting processes of in the organism is predefined by insufficiency of pumping function of myocardium, by deceleration of blood stream in the vessels of greater circulation, by uneven distribution of blood in the system of microcirculation [7].

Table 1. Rates of left ventricular diastolic function in patients with IHD and concomitant type 2 diabetes mellitus

Index	Control group, n=20	Group 1, n=39	Group 2, n=31	p_{1-2}
E, sm/sec	0.72±0.01	0.62±0.02*	0.56±0.02*	$p_{1-2}<0.05$
A, sm/sec	0.54±0.03	0.65±0.04*	0.70±0.03*	$p_{1-2}>0.05$
E/A	1.21±0.04	0.93±0.05*	0.79±0.04*	$p_{1-2}<0.05$
E', sm/sec	0.121±0.010	0.082±0.007*	0.060±0.006*	$p_{1-2}<0.05$
E/E'	5.34±0.18	7.81±0.12*	9.83±0.14*	$p_{1-2}<0.01$
DT, msec	172.12±3.02	181.26±5.23*	224.76±13.62*	$p_{1-2}<0.05$
IVRT, msec	80.14±1.32	98,04±4,25*	112.61±3.62*	$p_{1-2}<0.05$

Notes: 1. * – significant differences of indices in comparison with the control;
2. p_{1-2} – significant differences of indices between two groups of patients.

Table 2. Rates of arterial and venous blood oxygenation in patients with stable IHD, left ventricular diastolic dysfunction and type 2 diabetes mellitus

Index	Control group, n=20	1 group, n=39	2 group, n=31	p_{1-2}
$Sa.O_2$, %	99.10±0.41	96.12±0.74*	91.53±0.51*	$p_{1-2}<0.05$
$Sv.O_2$, %	70.31±0.33	67.26±0.41*	52.64±0.58*	$p_{1-2}<0.01$
$Ca.O_2$, ml·l^{-1}	149.20±0.62	142.10±1.53*	112.57±2.65*	$p_{1-2}<0.01$
$Cv.O_2$, ml·l^{-1}	98.33±0.62	94.14±1.41*	76.75±2.57*	$p_{1-2}<0.01$
$Da.O_2$-$Dv.O_2$, ml·l^{-1}	50.96±0.66	47.91±0.62*	43.85±0.94*	$p_{1-2}<0.05$

Notes: 1. * – significant differences of indices in comparison with the control,
2. p_{1-2} – significant differences of indices between two groups of patients.

For patients with stable IHD the circulatory hypoxia is of systemic nature, develops in the presence of chronic heart failure and develops in comorbid states [4, 7], type 2 diabetes mellitus in particular. It was established that in efficiency of oxygen transporting to the tissues of the organism, a functional ability of cardiovascular system is crucial [8], and that is why a search for effective methods of comorbid states treatment is a topical issue.

Conclusions

In the examined patients with stable IHD and concomitant type 2 diabetes mellitus more severe impairment of left ventricular diastolic function and the increase of its rigidity, caused by type 2 diabetes mellitus, were revealed.

In patients with stable IHD, left ventricular diastolic dysfunction and concomitant type 2 diabetes mellitus the levels of NT-proBNP and uric acid were significantly higher as compared to the patients with IHD without type 2 diabetes mellitus, which means that comorbidity with diabetes mellitus was the reason for more severe diastolic heart failure.

In patients with stable exertional angina and concomitant type 2 diabetes mellitus there was obvious oxygen deficiency in peripheral tissues with the decrease in oxygen volume consumed by the tissues of lower extremities, which worsens the course of chronic heart failure.

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CHOICE OF TREATMENT OPTIONS FOR CYSTIC LYMPHSTIC MALFORMATIONS IN THE HEAD AND NECK REGION: TREATMENT EXPERIENCE OF 81 CHILDREN

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Background. *Surgery has previously been the only treatment for lymphatic malformations (LMs), but in the head and neck region is challenging due to the risk of scarring, nerve damage, recurrence. Sclerotherapy may be a perfect alternative.*

Objective. *The aim of the study is to determine the efficacy and safety of the OK-432 sclerotherapy in the children with craniofacial LMs.*

Methods. *81 children with head and neck LM between December 2010 and March 2017were involved into the study. The follow-up period was from 6 to 79 months. According to the size of cysts, LMs were classified into macrocystic, microcystic, and mixed. The result of the treatment of LMs was determined by the percentage of reduction in size as excellent (decrease by more than 90%), good (by 50%–89%), satisfactory (by 20%–49%) and none (by less than 20%).*

Results. *The macrocystic LMs diagnosed in 41.97% of patients, microcystic – in 12.35%, and mixed – in 45.68% of children. OK-432 sclerotherapy only was performed for 83.9% of patients and in 12.3% in combination with surgery. The range of sclerotherapy sessions was from 1 to 11. An excellent result in 96.97% of cases was evidenced in the patients with macrocystic LM. Poor result was proved in the patients with microcystic LMs; the most of them (55.56%) had satisfactory result. In the patients with mixed LM, an excellent and good result was evidenced in 83.33%. After 198 sessions of OK-432 sclerotherapy, complications associated with the treatment occurred in 5 (2.52%) cases.*

Conclusions. *OK-432 sclerotherapy is a safe and effective treatment of head and neck LMs in children. Macrocystic LMs proved the best response to OK-432 treatment.*

KEY WORDS: **lymphatic malformations; sclerotherapy; OK-432; children.**

Introduction

Cystic lymphatic malformations are benign vascular lesions, which develop as the result of embryological disturbances of lymphatic system. Unfortunately, the terms often used to describe LMs are imprecise. 'Cystic hygroma' and 'lymphangioma' are often used incorrectly to describe malformations. Both of these terms should be abandoned, as the suffix *-oma* connotes a neoplasm [1]. In 1982, a landmark article by Mulliken and Glowacki suggested a classification system designating vascular anomalies as either tumours or malformations according to their biologic and pathologic features [2]. This system was adopted by the international society for the study of vascular anomalies (ISSVA) and was last updated in 2014 [3]. Vascular malformations are classified as capillary, venous, lymphatic, arterial, and mixed lesions, depending on their vascular tissue of origin. These lesions are present at birth and have a progressive clinical course. The incidence of LMs is evidenced in about 1 to 6000 to 1 to 16,000 live births, with a frequency of hospitalization – 3 cases per 100,000 [4, 5]. LMs near the principal lymphatic chains in the neck region are formed when a primordial lymph sac loses or fails to re-establish communication with the central veins (jugular and subclavian) from which it arises [1]. For many years, surgery has been the only treatment for LMs of any anatomical localization. However, the results of the operation were often disappointing, especially in cases of LM of cheek, tongue, oral cavity, neck lateral triangle. The surgical treatment of LM in the region of head and neck is accompanied by complications in 12–33% and recurrences in 15–53% of cases. In addition,

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resection of LM does not inhibit bones overgrowth in the region of LMs [6, 7, 8]. Recurrence and complications are usually the result of incomplete preoperative examination and incorrectly planned treatment. Incomplete resection provokes rapid growth of malformation, causes postoperative lymphorrhea, and recurrent infections [9]. The role of surgical treatment in order to achieve an excellent cosmetic result in patients with LMs is questionable. When planning an intervention, it is necessary to clearly understand that LM can sprout fascia, infiltrate different tissues and change normal anatomy. The damage of important nervous and vascular structures is unacceptable in the treatment of benign pathology [10]. LMs usually infiltrate skin and subcutaneous tissue [1], therefore after the operation the asymmetry progresses, and later lymph nodes can sprout post-operative scars.

Currently, there is no sufficient evidence in the literature to create treatment algorithms and help determine the best choice of initial therapy, and in recent decades sclerotherapy has become the most common treatment option [5, 11, 12].

According to the results of the literature analysis in the PubMed, Medline, Cochrane databases, three therapeutic agents are widely used for cystic LM local treatment: bleomycin, doxycycline and OK-432 (Picibanil) [13]. The features of endothelial cells lining the LMs cysts determine the effectiveness of the OK-432 for the treatment of LM. The mechanism of action of the drug is based on the induction of inflammation with subsequent activation of cytokines and apoptosis of cells that form the inner layer of the cysts [14]. In the cyst fluid the level of inflammatory mediators increases that confirms the inflammation in response to the injection of the drug [15]. The effectiveness of the OK-432 sclerotherapy in the treatment of cystic LMs has been described in small series of some medical centres [16, 17]. Since 2011 OK-432 is successfully used in Ukraine [18].

Objective of the study is to analyse the treatment experience of 81 child patients with lymphatic malformations (LM) in the head and neck region, and to determine the efficacy and safety of the OK-432 sclerotherapy in this cohort of patients.

Methods

During the period from December 2010 to March 2017, 126 patients with isolated LMs or LMs in combination with other congenital vascular malformations were treated in the OKHMATDYT National Specialized Children Hospital. The study includes 81 clinical cases of LMs that are localized in the head and neck region, representing 64.29% of all patients with LMs during the established period of time. The follow up period was from 6 to 79 months. There were 44 (53.7%) male and 37 (45.1%) female patients.

The following visualization methods were used to confirm the diagnosis of cystic LMs: ultrasound scan in grey-scale and colour Doppler was performed at the primary patient's examination and in dynamics, MRI was performed before treatment and at the stages of treatment, CT in urgent situation. According to the results of MRI, the size and structure of LMs and also their topographical relationship with neighbouring organs and tissues were determined as particularly mediastinum with retropharyngeal space involvement associated with LMs to the level of the hyoid bone. LMs were classified as macrocystic, microcystic, or mixed (macrocystic-microcystic), which has been proven useful in other sclerotherapy studies [19]. The classification was determined radiographically by MRI and ultrasound examination according to the cysts size: macrocystic (formed by cysts with a volume of more than 2 cubic centimetres (cc)), microcystic (cysts volume – less than 2 cc), and mixed, in which the microcystic component exceeds 50%. The size of LMs was determined using the ellipse square formula. The size of LMs up to 100 cm^2 was evaluated as small, 100–199 cm^2 – middle, 200–299 cm^2 – large and more than 300 cm^2 – gigantic. The result of the treatment of LM was determined by the percentage of reduction in the lesion size [3, 10] as excellent (decrease by more than 90%), good (50–89%), satisfactory (20–49%) and unsatisfactory (less than 20% reduction in size).

The statistical analysis was performed using IBM SPSS Statistics, version 23. The data is presented as an average of 95 percent confidence interval or standard deviation. The qualitative data (age, sex, localization, size, etc.) were analysed in a univariate analysis with the Pearson's correlation coefficient χ^2 test. The logistic regression model was used for variants that significantly predict the outcome of the treatment and/or the course of the disease. Statistical significance was defined as $p<0.05$.

Results

Diagnosis of LMs were established prenatally in 12 (14.81%) patients, at birth in 43 (53.08%),

during the first year of life in 12 children (14.81%), at the age of one to three years in 4 (4, 94%) and in 10 (12.35%) children, the clinical signs of the disease were manifested at the age above three years. Clinical signs of cystic LM in the head and neck region were: the asymmetry of face and neck due to mass in all children, compression of upper airway requiring tracheostomy before sclerotherapy (n=4, 3.6%), transient stridor during the first year of life (n=8, 7,1%), macroglossia, disarticulation (n=5, 4,5%), disturbance of occlusion (n=6, 5,4%), recurrence lymphorrhoea in the children with mucosal and skin lesions (n=4, 3.6%), excessive salivation (n=3, 2.7%), visual impairment (n=1, 0.9%), lymphedema of upper limb (n=1, 0.9%). 68 patients were treated primary, 13 children underwent surgical interventions previously. There patients were treated conservatively with propranolol within 6–12 months without any clinical result. According to the visualization of LMs using ultrasound and MRI, the topographic features of LM and relationship to adjacent organs were established. The lesions were localized in suprahyoid region in 37 (45.68%) children, and in infrahyoid region – in 44 (54.32%) cases. Bilateral mass were observed in 23 (28.39%) children, in other patients LMs were localized on the one side of middle line. 34 (41.97%) patients had macro cystic LMs, in 10 (12.35%) children microcytic LMs were diagnosed, and in 37 (45.68%) children LMs were mixed. The size of LMs ranged from 23 to 517 $см^2$, in average 135.87 $см^2$. In 37 (45.68%) patients LMs were small; in 29 (35.80%) they were middle, in 7 (8.64%) LMs were large and in 8 (9.88%) LMs were gigantic. In 22 (2716%) children mediastinal involvement was diagnosed. In children with head and neck LMs sclerotherapy, surgical and combined treatments were performed.

Three patients (3.7%) underwent surgical resection of LMs for the following reasons: recurrent inflammation requiring re-hospitalization and antibiotic therapy (n=2), difficulty in diagnostic due to sudden onset and atypical course of the disease (n=1). Recurrence after surgery happened in one case. One patient had postoperative complication: transient paresis of facial nerve branch.

Giant LMs, which occupy several anatomical sites and infiltrate muscles, bones, cellular spaces deserved a particular attention. Bilateral LMs narrowed airway and tracheostomy was evidenced in 5 patients. In this series, decannulation was eventually possible in 4 patients after multiple sessions of OK-432 sclerotherapy. One patient with bilateral LMs, mediastinum, retropharyngeal space and lung involvement died due to septic complications. Combined treatment was performed for 10 (12.3%) children, which consisted of the following procedures: resection of LM, tracheostomy prior to treatment, injection of OK-432 into residual cyst cavity during and/or after surgery (n=5, 6.17%), thoracocentesis, pleural and mediastinal drainage in the patient with purulent mediastinitis with subsequent sclerotherapy after the elimination of complications (n=1), sclerotherapy and correction of scarring and tissue deformities (n=3), resection of tongue in the patient with pronounced macroglossia, disturbance of occlusion (n=1), resection of orbital part of LMs due to visual impairment and subsequent sclerotherapy (n=1). The most patients (n=68, 83.90%) underwent the OK-432 sclerotherapy as a single method of head and neck LMs. Patients received from 1 to 11 sessions of sclerotherapy, in average 2.44±2.21 sessions per patient. After 1 session of the OK-432 sclerotherapy, an excellent result was achieved in 24 (29.63%) patients, according to localization and structure, these were unilateral macro cystic LMs of neck. Involvement of the mediastinum and retropharyngeal space was not a contraindication to the sclerotherapy of LMs. In 18 children with mediastinal and retropharyngeal part of LMs an excellent result was evidenced in 5 (27.78%), good in 9 (50.0%), and satisfactory in 2 (11.11%) cases. However, in 1 case, the result was poor and in 1 case the result of the treatment was unsatisfactory.

After 198 sessions of the OK-432 sclerotherapy, complications associated with the treatment occurred in 5 (2.52%) cases: significant oedema with the need for hospitalization, puncture, and decompression of the cyst (n=4; 2.02%), and skin allergic reaction (n=1; 0.51%). Response data for macro cystic LMs were higher than in other types of LMs with an excellent result in 96.97% and good result in 3.03% of patients. In the patients with microcystic LMs, no positive result was evidenced, 33.33% of cases had a good treatment result, 55.56% were satisfactory and 11.11% had no results. In the patients with mixed LM, an excellent result was evidenced in 33.33% of cases, 50.0% of patients had good response to treatment, 11.11% – a satisfactory one, and 2.77% of patients had no result.

Primary efficacy endpoints were evaluated using Fisher's exact test. Covariates used in a

univariate analysis included: LM type (macrocystic, microcystic, mixed), laterality (unilateral versus bilateral), mediastinal and retropharyngeal involvement, prior treatment, age during the first treatment, gender. It was established, that the result of treatment was determined primarily by the type of LM and previous intervention: the best results were evidenced in the patients with macrocystic LMs ($p<0.005$), previous intervention reduced the treatment efficacy ($p<0.005$). Covariates that proved a significant association with the treatment result were used for logistic regression analysis, the probability of an excellent and good outcome for the patients with macrocystic LM in cases of no prior treatment was 86.6%.

Discussion

This article presents a relatively large experience of the treatment of cystic LMs in the head and neck region for a relatively short period of time (5.5 years). A peculiarity of this study is that for the first time in Ukraine a new approach to the treatment of LMs of challenging localization in children is presented. Treatment of head and neck LMs is accompanied by significant risks, therefore for children it is necessary to choose the most effective and safe way, until stronger evidence is present, the difference in complication rates is potentially the deciding factor in the choice the treatment options [13].

For decades, resection has been the mainstay for management of LMs. More recently, sclerotherapy has become the first-line treatment for head and neck lesions. Experienced surgeons welcome the ascendancy of sclerosant management. Although some LMs can be successfully resected, complications are expected and results are often disappointing.

One of the most safe and effective ways of cystic LMs treatment in children is the OK-432 sclerotherapy [5, 16]. The OK-432 (Chugai Pharmaceuticals, Tokyo, Japan) is a lyophilized powder of Streptococcus pyogenes (group A, type 3, Su strain) incubated with benzylpenicillin. The drug was developed in Japan in the late 1960s as an antitumor agent. Although therapy of OK-432 did not increase the survival of the patients with cancer, its efficacy was proved in pleurodesis in cases of malignant pleural involvement. According to these studies, in the late 1980s, Japanese authors [17] published the first results of using OK-432 as a safe and effective treatment for cystic LMs; in 1994, the authors published the results of treatment of 94 patients with LMs [19]. Since then, the method has become widely used far beyond the borders of the country [20, 21]. The OK-432 is not exactly a sclerosing agent, because it doesn't destroy vascular endothelium. The OK-432 induces apoptosis of lymphatic endothelium and local cellular inflammatory reaction [7, 16]. Recent studies have proved that the pathway of the OK-432 action within lymphangiomas is probably cellular and cytokine-mediated [7, 16].

Complications due to OK-432 management are temporary and predictable [22]. According to the results of the literature analysis, no systemic hematological, renal, hepatic or cardiac side effects were detected [23], which was confirmed in our study. The typical side effect of OK-432 sclerotherapy is an inflammatory response that is accompanied by local oedema and may be potentially dangerous in cases of an airway compromised by cervical LMs. In our series, in the patients with impaired mechanics of breathing, tracheostomy was performed prior to treatment. All of those children had signs of respiratory failure, tracheostomy as a preventive procedure was not performed.

Unfortunately, none of the suggested methods for cystic LM treatment in children guarantees the complete recovery for all patients [24]; however, the introduction of a minimally invasive treatment of LM significantly reduced the percentage of open surgical interventions and made it possible to differentiate treatment in each individual clinical case.

Conclusions

The OK-432 sclerotherapy is safe and effective treatment option of head and neck LMs in children. Macrocystic LMs prove the best response to the OK-432 treatment, previous intervention and increasing the part of microcystic component reduces the efficacy of treatment. Complications associated with the OK-432 treatment occurred in 2.52% cases.

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CHARACTERISTIC FEATURES OF MULTIPLE ORGAN FAILURE IN CASES OF PERITONEAL SEPSIS

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Background. *The study of hystomorphology of liver and small intestine in experimental peritonitis is presented. Due to this information the criterion of pathogenetic moment transition SIRS for peritoneal sepsis was determined.*

Objective. *The aim of the research was to study the morphology of terminal part of small intestine and liver in cases of experimental peritonitis.*

Methods. *For histological and electron microscope study the biopsy of liver, small intestine were taken; the samples were stabilized in a neutral formalin, dried in alcohol of increasing concentration and placed in paraffin. Paraffin sections were painted with hematoxylin and eosin and studied under the light-optical microscope.*

Results. *Apoptosis caused damage to enterocytes and hepatocytes of first bacterial translocation. Mechanism of vasodilatation effect of NO and its effect on apoptosis were determined. Septic shock was accompaniment of two main levels of body cells damage: apoptosis and membranes destruction. Peritoneal sepsis is a grave condition caused by progressive peritonitis and polyorgan insufficiency syndrome. The phases of peritonealis sepsis pathogenesis were defined.*

Conclusions. *The presented morphological criteria prove the initiation of apoptosis within 24 hours after the development of peritonitis in enterocytes and hepatocytes.*

KEY WORDS: **peritonealis sepsis; apoptosis; polyorgan insufficiency syndrome.**

Introduction

Twenty-five years ago at the Chicago Consensus Conference on Sepsis some traditional approaches and postulates as well as critical approach to the suggested concept has been changed [1, 2].

The accumulation of experimental and clinical data on the significance of cytokines in the pathogenesis of sepsis allows adequate formulation of clinical diagnosis and formation of accurate subject [5, 7].

The realization of systemic inflammatory response syndrome (SIRS), as a part of diagnosis that characterizes the septic state of patients, allowed building algorithm due to pathogenic effects.

At the same time, much criticism by the supporters of the traditional concept of 'sepsis as a bacteremia accompanied by appropriate clinical disruption of homeostasis' that are inherent to SIRS substantiate that clinical signs of the syndrome are a common reaction to surgical infection.

In our clinic, the researchers believe that the determination of septic states is not reasonable enough: sepsis, severe sepsis, septic shock, hypotension syndrome and multiple organ failure.

The concept peritoneal (abdominal) sepsis is even more terminologically confusing.

Our aim was to define the morphological criteria, which determine pathogenic moment of SIRS transition that causes peritoneal sepsis at peritonitis.

Methods

During the research the morphology of terminal part of small intestine and liver in experimental peritonitis was studied.

The research took place in spring. 20 mature nonlinear white male rats, weighing 200–250 g were used. The animals were kept on a standard vivarium diet with free access to water in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" [6, 9].

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Acute peritonitis was modeled by administration of 10% fecal mixture into abdominal cavity by the method of Lazarenko V. A., et al (2008) [8] that is comparable to etiological factors, clinical manifestations and phase transition of similar process in humans and allows the death of animals, which is acceptable for conducting a dynamic study during 10 days. This was performed by the administration of 0.5 ml of 10% fecal suspension filtered into peritoneal cavity of the studied rats. The suspension was obtained by mixing isotonic solutions and feces from the intestine of two or three intact animals and then it was filtered twice through a double layer of gauze. The resulting suspension no later than in 20 minutes after preparation was administered to the intact animals by puncture method. To avoid damage to internal organs when the fecal suspension was introduced into abdominal cavity, the animals were held upright, caudal end up. By the method of puncture of ventral wall in the middle of the central line of abdomen, the end of the needle was rotated in turn into the right and left hypochondria, the right and left ilium sections, and the same amount of fecal suspension was injected. In 24 hours the animals were killed by decapitation under general anaesthesia.

For histological and electron microscope study biopsies of liver, small intestine were taken. The samples were stabilized in neutral formalin, dried in alcohols of increasing concentration and placed in paraffin. Paraffin sections were painted with hematoxylin and eosin and studied under the light-optical microscope.

Electron microscope examination of biopsy samples was processed by standard methods and placed in epoxy resin. In addition, the method was used to identify membrane permeability due to Ca^{++}-ATP. Thick ultrathin sections 600 Å were managed in ultra-microtome LKB and Reyhert. To improve the contrast the sections were painted by Reynholds and studied through the electron microscope EM-400, Philips.

Results

Histological examination of small intestine wall in all animals, which were simulated with diffuse peritonitis, proved dystrophic and destructive changes of all its layers, mainly affecting submucosal tissues: oedema on the background of disturbed intra-organ blood flow was the most pronounced that was manifested with plethora expansion and main mass of microvessels, perivascular and interstitial haemorrhage. Associated with oedema, histo-architectonic connective tissue stroma was damaged. Mucous membrane was sometimes flaky in intestine and a large mass of microvilli was swollen and deformed. Epithelial vessels microvilli were swollen with a clarified cytoplasm and basophilic nucleus was eccentrically placed. Muscle shell was also swollen with more extended and filled with blood microvessels, as a result focal violations moved muscle fibres with expansion to intramuscular spaces.

The electron microscope study of columnar epithelium intestinal mucosa proved pronounced dystrophic and destructive changes of nucleus and cytoplasm. Nuclei fracture, chromatin aggregation and redistribution of its overwhelming pre-shell condensation were established; normal chromatin in the nuclei of epithelial cells of intestine was placed uniformly in plasma nuclei.

In the cytoplasm of enterocytes intracellular accumulation of detritus was evidenced, proving fairly pronounced destructive changes in these cells. Changes occur in the microvilli system that protrude the intestine in a gleam. Histochemical reaction on membrane permeability using the Ca^{++}-ATP-ase proved that compare to normal, reaction products fell in the sediment on the surface membranes of microvilli and determined in their gleam penetrating into the cytoplasm of cells. This evidenced membrane permeability microvilli violation associated with their swelling.

External intestinal serosa studied on light-optical level was slightly thickened and swollen. Violations of connective tissue skeleton swelling and disorientation of collagen and elastic fibers and desquamation mesothelial cells were also noticed. Numerous cavities extended microvascular events with accumulation of red blood cells and endothelial swelling. In the parietal layer of peritoneum the changes were less pronounced and comprised extended microvessels cavity and perivascular connective tissue swelling (Fig. 1.)

This resulted in pathological permeability of intestine inner lining and was accompanied by increased penetration of endotoxin, bacterial contamination from intestine cavity into mesenteric lymph nodes and portal system.

Microscopic examination of liver in all cases proved more or less pronounced swelling of parenchyma on the background of disturbed intra-organ blood flow, which was manifested by plethora expansion and main vessels mass-

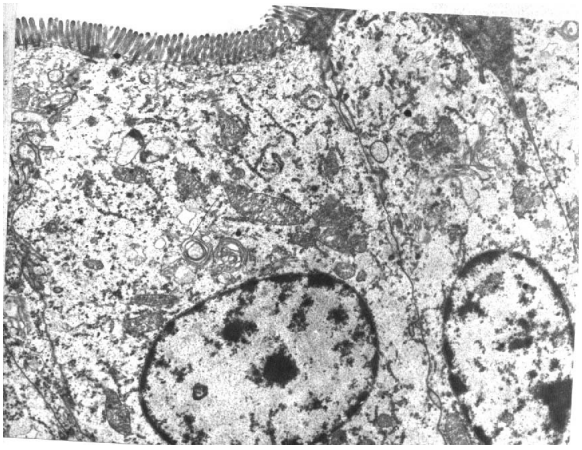

Fig. 1. Modeling of acute peritonitis. Oedema and violation of intracellular organelles integrity of intestine epithelial cells. Electron micrographs ×13000

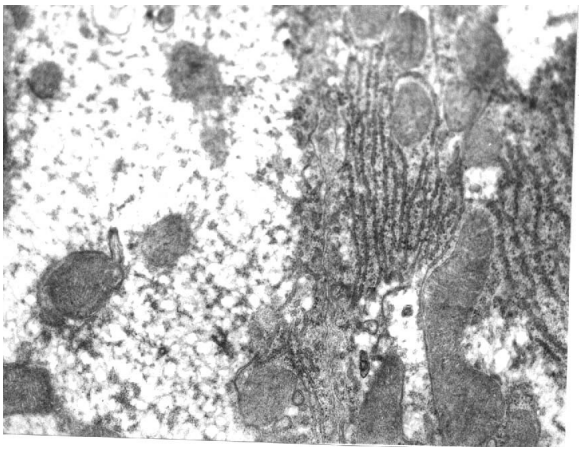

Fig. 2. Modeling of acute peritonitis. Oedema of hepatocyte with violation of mitochondria integrity and reduction of endoplasmic reticulum. Electron micrographs ×13000

es and focal perivascular haemorrhage. In areas of disturbed intra-organ blood flow quite pronounced swelling of liver cells with impaired liver histological structure and expanding intercellular spaces was evidenced. The investigation of hepatocytes structure in a significant increase in a series of semi-thin slices proved pronounced degenerative changes of liver cells with sharp cytoplasm enlightenment, significant decrease in the size of the nucleus, associated with swelling, decrease in the number and pre-shell condensation of chromatin. In addition, significantly increased sinusoids with focal desquamation of endothelial cells that route to the cavity of blood vessels was observed.

Electron microscopic examination of hepatocytes proved that some of these cells had pronounced degenerative changes associated with swelling of cytoplasm, manifested by quite significant expanding and vacuolization of endoplasmic reticulum, mitochondria and destruction of the significant decrease in the number of free and fixed ribonucleic granules. Ultrastructural changes were detected in endothelial cells of hepatic sinusoid. These changes were of diverse nature: from dystrophic-atrophic to hyperplastic, proving the focal reparative effects. In hepatocytes that were adjacent to hyperplastic endothelial cells the effects reparative regeneration were established that comprised strengthening their energy-producing and protein synthesis function by hypertrophy of mitochondria and increase in the number of free ribosomes and polysomes (Fig. 2.).

Thus, the presented morphological criteria proved the initiation of apoptosis in enterocytes and hepatocytes within 24 hours after the development of peritonitis.

Discussion

Kerr [2] described these effects in 1972 as 'apoptosis' cause, we believe that further destruction of cellular organelles took place. Thus, in most enterocytes the damaged mitochondria, swollen and infected reticulum cisterns with a reduced number of ribonucleic granules fixed in their membrane were evidenced. The number of ribosomes and polysomes was reduces.

According to the literature, the mentioned processes cause local activation of immune cells, cytokines and other inflammatory mediators (TNF, IL-1,6,8), which are able to stimulate the production of prostaglandins, free radicals, nitric oxide, which in turn is a powerful factor in adaptive processes at a cellular level [10, 11].

The destruction of liver cells during peritonitis occurs also due to apoptosis. It is likely that this process is initiated by nitric oxide, which in living organisms is formed by the action of specific enzymes united under the title of nitric oxide synthase. The last were found in endothelial cells of arteries and veins of intestine, and were initiated by the generation of nitric oxide in all cells of liver [3, 4].

These changes may be enough to form multiple organ failure, especially such as bacterial translocation. In our opinion, the appearance of morphological, biochemical and clinical signs of organ failure should be the criteria for diagnosis 'peritoneal sepsis' (Fig. 3.). Further development of the pathological process is determined by two-way cell destruction: by apoptosis and degradation of membranes, and can lead to septic shock. If at this moment intensive multicomponent therapy does not help stabilize homeostasis, the irreversible changes

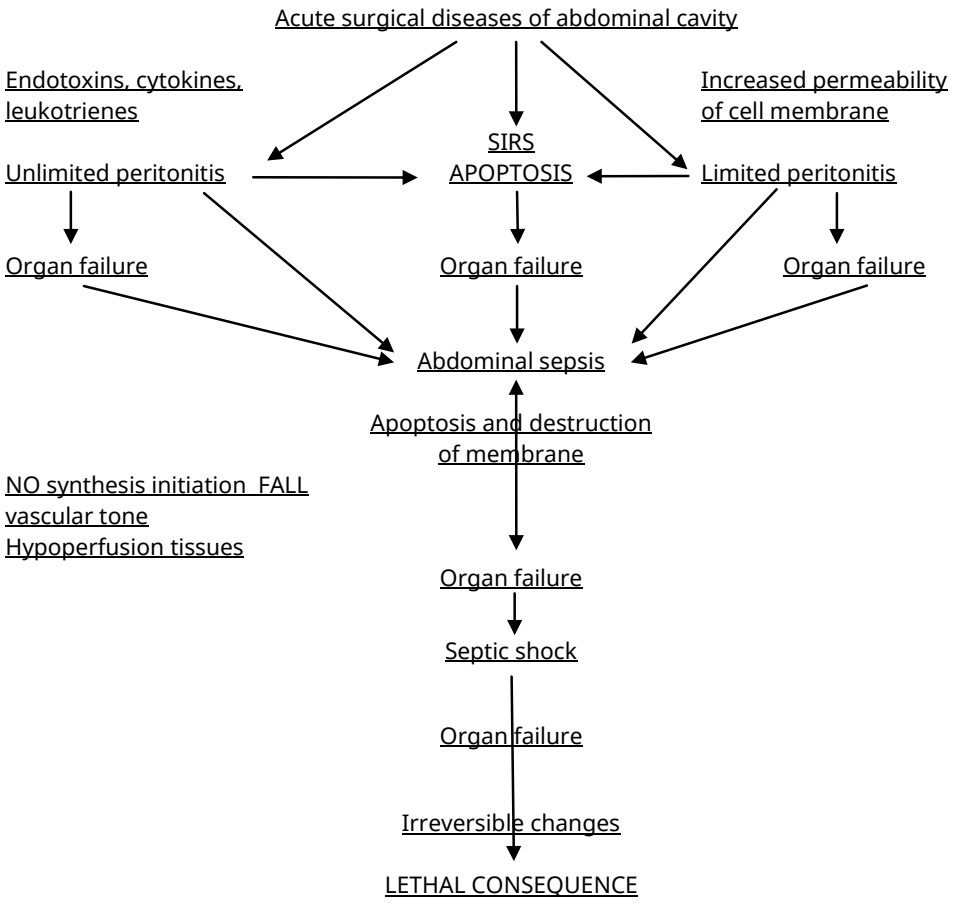

Fig. 3. Pathogenetic variants of peritoneal sepsis

in vital organs and systems will take place that may lead to fatal consequences.

We understand that the above concept is hypothetical and disputable to some extent, but morphological changes prove the need for further study of the problem, especially the determination of nitric oxide, its synthase and apoptosis.

Conclusions

The affection of hepatocytes and enterocytes in experimental peritonitis begins with apoptosis.

Apoptosis, probably, can be morphological criteria of multiple organ failure initiation.

Peritoneal sepsis is a severe pathology resulting in progression of peritonitis and multiple organ failure.

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ANTENATAL INVESTIGATION OF DUCTUS VENOSUS VELOCITY AS A METHOD OF DETECTING THE FETAL HEART FAILURE, CAUSED BY PARVOVIRUS B19 INFECTION

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Background. *The article describes the methods and results of investigation of blood flow velocity waveforms in fetal ductus venosus (DV). These studies are used to visualize the degree of fetal heart failure and determine its further clinical course.*

Objective. *The study was aimed to predict the development of heart failure in the fetuses that were infected with parvovirus B19 infection during 11-14 gestation weeks by measuring the Doppler parameters of blood flow velocity in the DV.*

Methods. *Our investigation involved 20 pregnant women aged from 18 to 30 years old who were infected with parvovirus B19 infection during the period from 11 to 14 weeks of gestation. The DV was determined by means of color Doppler. Fetal echocardiography (EchoCG) was performed by means of the ultrasound scanner Philips HD IIXE device (USA) using a transabdominal convex probe with the frequency of 3.5 MHz, operating in a CDC mode and the frequency filter at 100 Hz. The A-wave directivity evaluation in the DV was investigated according to the Guideline Principles of the Fetal Medicine Foundation (www.fetalmedicine.com). Statistical processing of data was carried out using the package of applied programs Microsoft Office Excel 2016 and Statistica 6, Stata 12.*

Results. *In 16 of 20 (80%) fetuses we did not observe any absent or reversed A-wave flow in the DV during atrial contraction as well as any fetal echocardiographic pathological signs. In 2 (10%) cases a reversed A-wave flow in the DV in a combination with EchoCG-signs of overload of left side of heart, resulting in enlargement (dilatation) of left atrium and left ventricle were detected. In 2 (10%) cases the presence of a reversed A-wave flow in the DV and EchoCG-signs of fetal heart failure (reduction of cardiac output, significant dilatation of left ventricle) were evidenced.*

The results of the study confirm that with the expansion of fetal nuchal translucency thickness, the systolic blood flow velocity in the DV increases with the correlation coefficient r=0.594, which proves a linear dependence between these two ultrasonography parameters.

Conclusions. *The linear correlation between the presence of a reversed A-wave blood flow velocity in the DV and the overload of left side of fetal heart, development of heart failure (20% of the total number of examined women) were proved.*

KEY WORDS: **ductus venosus (DV); heart failure; parvovirus B19 infection; peak systolic blood flow velocity; fetal echocardiography (EchoCG).**

Introduction

Parvovirus B19 infection is a potentially life-threatening disease [1] for a fetus, especially during the first two trimesters of pregnancy that can cause a variety of signs of its damage (severe anemia, ventriculomegaly, hypertrophic myocardiopathy and pericardial effusion, non-immune fetal hydrops, hydropic or nonhydropic intrauterine fetal death, intrauterine growth retardation, thrombocytopenia, meconium peritonitis, hepatic calcifications, abnormal long-term neurodevelopment, ascites, placentomegaly, etc.) [2]. The risk of fetal death depends on gestational age in cases of infection. According to gestational age, maternal infection in the first trimester leads to fetal death in 19%, in 13–20 weeks – 15% and after 20 weeks – 6% of cases [3]. It is quite widespread. Approximately 1–5% of women is susceptible to parvovirus and develops serologic evidences during pregnancy period; the frequency of morbidity is rising to 3-34% in epidemic periods; about 50% of women have an asymptomatic course of the disease [4].

Infection with parvovirus B19 affects many organs and systems, mostly cardiovascular system. The transmission rate of maternal parvovirus B19 infection to fetus is 17% to 33%

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[5]. Transplacental viral transmission is probably explained by the presence in villous trophoblastic cells of placental tissues the maximum level of neutral glycolipid known as blood group antigen P (globoside), which also serves as a receptor for B19, during the first two trimesters of pregnancy. Cardiomyocytes are the most important target cells for parvovirus. P-antigen expressed on fetal cardiac myocytes enables the virus to infect myocardial cells and induce myocarditis that aggravates cardiac failure. Fetal heart failure may be caured by severe anemia but may often be associated with myocarditis, which can cause arrhythmias or even cardiac arrest without evidence of anemia, cardiac failure or hydrops. Infection within myocardium often results in acute inflammation, but may also lead to non-inflammatory damage of cells, and as a consequence, to infection-related cardiomyopathy. Virus B19 enters the endothelium cells utilizing a globoside and its co-receptors a5 pi-integrin and Ku80 and after connection with a receptor pass to the state of persistent infection in the endothelium of various organs, including heart. In consequence, numerous copies of the viral genome of B19 are determined in the endothelium of intramyocardial arterioles, capillaries and post-capillary venules, initiating inflammation process induced by the continuous expression of pro-inflammatory cytokines (TNF-a, interleukins (IL): IL-6, IL-8, IL-2)), IgG, soluble interleukin 2 receptor, leukotrienes and prostaglandins [6]. The direct cytopathic effect of parvovirus, apoptosis, activation of innate and adaptive immune response lead to endothelial dysfunction, followed by ventricular remodeling and development of dilated cardiomyopathy.

The asymptomatic course and late detection of parvovirus B19 infection during antenatal period are two real problems which may lead to the development of cardiac fetal insufficiency.

The advanced ultrasound allows characterizing many complex conditions which are necessary to evaluate and understanding various pathologies that contribute to heart failure in fetus. Doppler ultrasound tools have improved our understanding of fetal circulation and patho-physiological mechanism that controls fetal circulation. According to the literature, ductus venosus (DV) Doppler measurements can give information on pregnancy courses, its prognosis and can therefore be recommended as a part of the routine workup of pregnancies, complicated by viral infections, such as parvovirus B19.

The article describes the methods and results of investigation of blood flow velocity waveforms in fetal DV. These studies are used to visualize the degree of fetal heart failure and determine its further clinical course.

The aim of our research was to predict the development of heart failure in the fetuses that were infected with parvovirus B19 infection during 11–14 gestation weeks by measuring the Doppler parameters of blood flow velocity in the DV.

Methods

20 pregnant women, who were infected with parvovirus B19 during the period from 11 to 14 weeks of gestation, were involved into the study. The inclusion criteria were: age from 18 to 30 years old; singleton pregnancy; absence of extragenital pathology events in the sub- and decompensation stages; gestation period from 11 to 14 weeks; no episode of a threatened abortion during pregnancy; absence of fetal chromosomal abnormalities. The DV was identified by means of color Doppler. Given that its localization at the cephalic end of intraabdominal portion of umbilical vein we used two approaches in an oblique transverse and sagittal imaging planes through the fetal abdomen to visualize the communication between the umbilical vein and the inferior vena cava. The presence of characteristic high-velocity signals in the color Doppler B-mode proved the identification. Our record was taken in the inlet of DV with an insonation ange as near to the long axis of the vessel as possible. Blood flow patterns merely proved changes in the pressure between the vessel and the right atrium throughout the cardiac cycle. After activating the color Doppler using a low velocity setting (<0.24 m/s), three vessels were detected in cross-section: the abdominal aorta, the inferior vena, and the DV. Unlike the superior and inferior vena cavae in which there may be a reverse flow during atrial systole, the flow through the DV proves continuous forward flow towards the heart. Normal flow in the DV was low velocity and triphasic consisting of maximal velocities during ventricular systole (S -wave) in relation with a rapid filling of the atria; the second peak (D-wave) corresponded to the early ventricular diastole, and the flow velocity was minimal during atrial contraction (A-wave). The A-wave directivity evaluation in the DV was investigated according to the Guideline Principles of the Fetal Medicine Foundation (www.fetalmedicine.com). The waveforms were clas-

sified as normal or abnormal depending on whether the A-wave lowest forward velocity during atrial contraction in late diastole was positive or absent/reversed, respectively. Absent or reverse A-wave in the DV proved fetal cardiac failure. Fetal echocardiography (EchoCG) included assessment of the position of heart, the four-chamber view, the outflow tracts and the venous return to the heart. EchoCG was performed by the ultrasound scanner Philips HD II XE device (USA) using a transabdominal convex probe with the frequency of 3.5 MHz, operating in a CDC mode and the frequency filter at 100 Hz. Statistical processing of data was carried out using the package of applied programs Microsoft Office Excel 2016 and Statistica 6, Stata 12. Statistically significant differences at $p<0.05$ were considered.

Results

Analyzing the results of investigation, in 16 of 20 (80%) fetuses we didn't observe any absent or reversed A-wave flow in the DV during atrial contraction as well as any fetal echocardiographic pathological signs of cardiac damage (Fig. 1). In 2 (10%) cases the reversed A-wave flow in the DV in a combination with EchoCG-signs of overload of left side of heart, resulting in enlargement (dilatation) of left atrium and left ventricle were detected. In 2 (10%) cases the presence of reversed A-wave flow in the DV and EchoCG-signs of fetal heart failure (reduction of cardiac output, significant dilatation of left ventricle) were evidenced (Fig. 2).

In 20% of the examined women (4 cases) with the presence of reversed A-wave blood flow velocity in the DV we also made a mathematical correlation between the peak systolic blood flow velocity in the DV and the fetal nuchal translucency thickness, as one of the EchoCG markers of fetal heart failure. The findings confirm that with the expansion of fetal nuchal translucency thickness there is an increased peak systolic blood flow velocity in the DV with the correlation coefficient r=0.594 (the coefficient of determination is R2=0.3534 with normal range r=0.161 (R2=0.0258)) that proves a linear dependence between these two ultrasonography parameters (Fig. 3): the coefficient of determination is the percentage of response variable variation that is explained by a linear model (dependence on the changes of one parameter from another in percentages) – in our case 0,3534 (35,34%) which means, that the peak systolic blood flow velocity changes in the DV 35.34% depends on changes in the fetal nuchal translucency thickness and could predict the future fetal cardiac compromise ($p<0.05$).

Discussion

Dynamic observation of gestational process allows relate some features of ultrasound pattern evidenced in 11–14-week fetuses with an increased risk of heart failure development. Parvovirus B19 infects primarily the erythroid cell line and may therefore cause fetal anemia and consequently cardiac failure with hydrops fetalis in some cases. Doppler measurement technics today can modify treatment so that the number of fetuses infected with virus B19 and its attendant risks can be minimized, without missing the opportunity for timely, life-saving intervention when it is warranted.

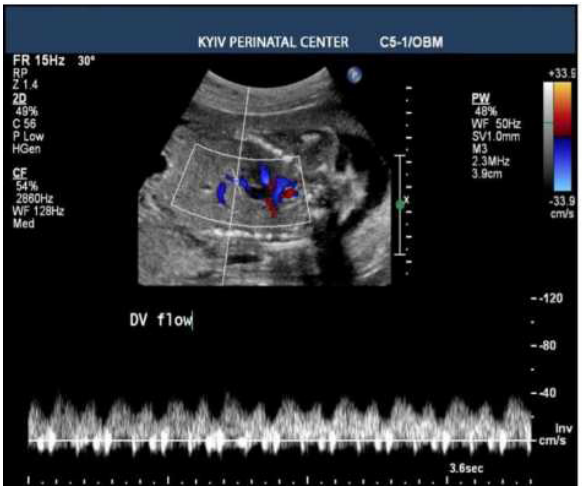

Fig. 1 (leftward). Normal blood flow velocity measured in the ductus venosus during 12 gestation weeks in the woman infected with parvovirus B19

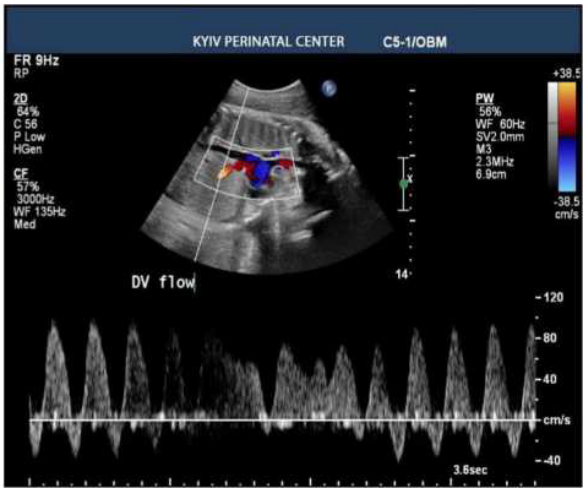

Fig. 2 (rightward). Reversed A-wave flow waveform in the ductus venosus during 12-13 gestation weeks measured in the woman infected with parvovirus B19

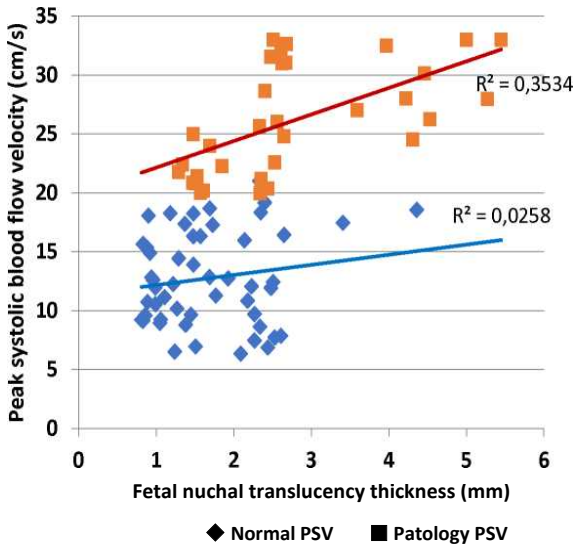

Fig. 3. Correlation curve between the peak systolic blood flow velocity in the ductus venosus (cm/s) and fetal nuchal translucency thickness (mm)

Increased fetal nuchal translucency thickness was established to be a myocardial dysfunction and/or fetal anemia [7]. The correlation between the increased nuchal translucency and the presence of abnormal blood flow profile in the DV was proved. The measurement of peak systolic blood flow velocity in the DV is the latest tool of non-invasive monitoring of fetuses at risk for anemia and cardiac insufficiency due to parvovirus B19. In the article, the increased fetal nuchal translucency thickness was found with reversed flow in the DV during atrial contraction, this findings were helpful indicators of the presence of fetal heart failure.

In summary, the linear correlation between the presence of a reversed A-wave blood flow velocity in the DV and the overload of left fetal heart, the development of heart failure (20% of the total number of examined women) was proved.

Conclusions

Parvovirus B19 infection has a direct pathological effect on the endothelium of intramyocardial arterioles, capillaries and post-capillary venules. The development of dilated cardiomyopathy and heart failure are two pathological consequences of B19 cardiac damage.

The most reliable way to diagnose fetal heart failure during antenatal period is the Doppler and EchoCG investigation of blood flow velocity waveform in the DV and the measurement of nuchal translucency thickness.

In case of left-sided heart insufficiency occur the volume overload of right side of fetal heart with increased pressure in the DV, the presence of a reversed A-wave flow velocity in the DV and increased peak systolic blood flow, the expansion of fetal nuchal translucency thickness and decreasing left ventricular isovolumic relaxation time.

A linear relationship between the increased fetal nuchal translucency thickness and the peak systolic blood flow velocity in the DV was determined.

According to the results of the study, we can conclude that there is a direct correlation between the appearance of a reversed A-wave blood flow in the DV and the development of fetal heart failure.

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BIOMETRIC METHOD OF AGE ESTIMATION: DEVELOPMENT AND EFFICIENCY, IN CASES OF PATHOLOGIES OF TEETH HARD TISSUES

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Background. *The physiological changes of tooth are the criteria used in evaluation of regressive formula by Kvaal et al. age estimation technique. But in cases of abnormal occlusion, abnormal chewing habits, bruxism, abrasive factors or structural defects of teeth the intensity of tooth aging accelerates.*

Objective. *The aim of the research was to define the options of age estimation according to dental state of individuals with pathological attrition.*

Methods. *108 panoramic x-ray photos of patients with pathological attrition of teeth were chosen by a randomized selection (49 males and 59 females). All photos were made by means of Planmeca PROMAX orthopantomograph. Nine measurements were made for each tooth: the tooth length, pulp length, root length, root width and pulp width at three different levels: cement-enamel junction (level A, beginning of root), one-quarter of root length from a cement-enamel junction (level B), and mid-root (level C). Due to these measurements, a number of ratios were calculated in accordance with Kvaal et al. method.*

Results. *The errors that reached 27±8.4 years were found when evaluating the dental age using primary coefficients of equations suggested by the authors of the method used. By means of mathematical analyses, principal component regression method as well, the correlation coefficient of Pearson and method of combining linear regression due to the tooth changes in cases of pathological attrition (lowering level of occlusal surface, dystrophy of pulp structures and deposition of tertiary reparative dentine) by regression analysis, the modified formulas for age estimation using radiographic technique were found. Modified coefficients decreased the error to 13±0.8 years, which was relative to the real age upto nearly 42–48% compared to the primary coefficients of equations for pathological attrition.*

Conclusions. *Age estimation technique can be improved taking into account morphological changes in pathological attrition and the calculated coefficients make it possible to expand the circle of person's age which needs to be found.*

KEY WORDS: **age estimation; Kvaal method; pathological attrition; regression analyses; reparative dentine deposition.**

Introduction

Estimation of biological age of a person is significant in forensic science, especially for comparative and reconstructive identification antemortem and postmortem as recommended by Interpol/ICPO (International Criminal Police Organization) and FBI (Federal Bureau of Investigation). Justice bodies in an ethnically heterogeneous society use the results of age estimation by dental status where age indicators affect the need of socio-vulnerable persons, illegal immigrants and children and allow benefiting from the state budget; it also influences on level of criminal responsibility of persons with regard to age limit [1]. Age is the least variable and probably the most accurate determining parameter, since the aging process is the most independently reflected in changes of pulp and hard tissues of teeth than any other functional system of the body that is more vulnerable to the effects of pathologal features, constitution and physiological defects. Practical determination of the age of adults is possible using morphological techniques of Gustafson G. ("Age determination on teeth"), Bang G., Ramm E. ("Determination of age in humans from root dentine transparency") Johanson G. ("Age determination from teeth"), Maples W. R. ("An improved technique using dental histology for estimation of adult age"),

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and morphologically-radiographic techniques of Solheim T. ("A new method for dental age estimation in adults"), Kvaal S. I. et al ("Age estimation of adults from dental radiographs") [2–10]. The most rational method which excludes extraction of teeth and subjective grading of morphological indicators is Kvaal et al. technique, which involves calculating the ratio of length of crown and root to the length of pulp, width of root to the width of pulp in specifically designated areas, searches averages and uses standardized coefficients for the final result. However, this technique does not provide the effective use in cases of the presence of hard tissue lesions of teeth, pathological attrition is the most common. Attrition is a constant form of retrogressive changes in teeth, which involves lowering the level of occlusal surface in the amount related to the normal process of aging. The physiological loss of hard tissue caused by tooth-to-tooth contact in occlusion and mastification depends on diet, dentition, force of mastificatory muscles and chewing habits. Physiological attrition is proportional to the age of an individual as deposition of secondary dentine or pulp changes during lifetime [11–13]. The physiological changes of tooth are the criteria used in calculation of regressive formula by Kvaal et al. age estimation technique. But in cases of abnormal occlusion (crowding of teeth, malposed teeth, lesions of prosthetics treatment), abnormal chewing habits, bruxism, abrasive factors or structural defects in teeth take place the intensity of tooth aging increases. This process is pathological attrition. We have found out that in pathological attrition the occlusal level of teeth lowers in a few times faster in dependence of forces that influence. Formation of reparative tertiary dentine, closing volume of pulp chamber and dystrophy processes take place in pulp structure that is unusual for physiological attrition [14–20]. Due to all these factors and principles we have approbated primary method of Kvaal et al. age estimation technique and found modified regression formulas that approximate the calculation results with the real age.

Methods

108 panoramic X-rays photo of patients with pathological attrition of teeth were chosen by a randomized selection (49 males and 59 females). All photos were made by means of Planmeca PROMAX orthopantomograph [14–15]. Using graphical redactor Adobe Photoshop CS3 some teeth were cut from each panoramic photo: maxillary central incisor, lateral incisor and second premolar, mandibular later incisor, canine and first premolar, and all of them were positioned strongly on vertical axis. Nine measurements of each tooth were made: tooth length, pulp length, root length, root width and pulp width at three different levels: cement-enamel junction – (level A, beginning of root), one-quarter of the root length from the cement-enamel junction (level B), and mid-root (level C) [10]. All measurements were performed by means of Measurement tool in Adobe Photoshop CS3 mainly in pixels and then converted into millimeters. For each value 7 measurements were made and for further calculation the average means were estimated (Table 1).

From the measurements, a number of ratios were calculated in accordance with Kvaal et al.: P – the ratio of pulp length to root length; T – the ratio of tooth length to root length; R – the ratio of pulp length to tooth length; A – the ratio of width of pulp to root at level A; B – the ratio of width of pulp to root at level B; C – the ratio of width of pulp to root at level C; M – the mean values of all ratios; W – the mean value of width ratios from levels B and C; L – the mean value of length ratios *P* and *R*; W-L – the difference between *W* and *L [10]*. All ratios were calculated using standard Microsoft Office program package Microsoft Office Excel. The mean of all ratios (*M*) was used as the first predictor, while the difference between the mean of the 2 width ratios and the mean of the 2 length ratios (*W-L*) was used as the second predictor. The results of estimated age differ significantly from real age and reach the error up to nearly 48%. Using correlation coefficient and principal component regression method it was established that the strongest correlation between age results of patients with pathological attrition and calculated ratios was evidenced between R and further L, W, and also A (as the level closest to the centers of reparative dentine formation), which occurred because of significantly different level of tooth loss and closing volume of pulp chamber due to attrition (Table 2).

The statistical information was analyzed by means of Statystics Pro software and linear regression analysis, thus new modified coefficients for Kvaal et al. primary formulas were estimated (Table 3).

As a result, the error reached 13±0.9 years and did not increase more.

Table 1. Example of measurement of tooth specific indicators of patient with pathological attrition

Specific parameters	Base/repeat measurements	Measurements and calculations		
		Number of measurements	Mean (mm)	Difference (mm)
Tooth length	Main measurement	7	22.07	0.03
	Repeated measurement	7	22.10	
Pulp length	Main measurement	7	17.02	0.47
	Repeated measurement	7	16.55	
Root length	Main measurement	7	15.00	0.29
	Repeated measurement	7	14.71	
Pulp width A	Main measurement	7	0.82	0.01
	Repeated measurement	7	0.81	
Pulp width B	Main measurement	60	0.72	0.02
	Repeated measurement	60	0.70	
Pulp width C	Main measurement	60	0.39	0.01
	Repeated measurement	60	0.40	
Root width A	Main measurement	60	5.14	0.04
	Repeated measurement	60	5.10	
Root width B	Main measurement	60	4.36	0.07
	Repeated measurement	60	4.29	
Root width C	Main measurement	60	3.82	0.07
	Repeated measurement	60	3.75	

Table 2. Correlation between age of patients with pathological attrition and the ratios of measurements

	Upper central incisor	Upper lateral incisor	Upper second premolar	Lower lateral incisor	Lover canine	Lower first premolar
P	-0.11	-0.08	-0.16	-0.15	-0.07	-0.49
T	-0.34	-0.07	-0.11	-0.12	-0.16	-0.44
R	0.24	-0.14	-0.16	-0.12	-0.04	-0.28
A	-0.19	-0.30	-0.16	-0.22	-0.90	-0.10
B	-0.30	-0.20	-0.16	-0.32	-0.14	-0.20
C	-0.32	-0.30	-0.27	-0.31	-0.15	-0.20
M	-0.31	-0.26	-0.21	-0.34	-0.17	-0.39
L	-0.08	-0.11	-0.17	-0.27	-0.14	-0.23
W-L	-0.39	-0.14	-0.08	-0.30	-0.02	0.21

Table 3. Modified regression equations of patients with pathological attrition

	Equation	R2 (Coefficient of determination)	Significant predictors
All six teeth	Age=45.1+5.42 M+3.76 W-L	0.21	None
Lower canine	Age=77.1–84.1 M–51.09 W-L	0.012	None
Lower lateral incisor	Age=24.6+4.06 M–19.01 W-L	0.04	None
Lower first premolar	Age=–21.4+16.5 M–36.1 W-L	0.356	M and W-L
Upper second premolar	Age=125.6–84.02 M+42.4 W-L	0.211	W-L
Upper lateral incisor	Age=35.11–16.5 M–38.1 W-L	0.214	None
Upper central incisor	Age=35.6–76.8 M–56.3 W-L	0.27	W-L
Upper three teeth	Age=30.14+14.7 M+2.10 W-L	0.045	M
Lower three teeth	Age=19.2+5.7 M–12.18 W-L	0.051	W-L

Results

The most significant correlation results between tooth and age were established in upper and lower incisors, and lower premolar. The lowest correlation was found at lower canine in patients with pathological attrition because of the level of influence of pathological attrition on different types of tooth. The Pearson correlation coefficients between chronological age and different ratios (P, T, R, A, B, C) calculated due to the length and width measurements on the orthopantomographs are displayed in Table 2. The differences compared to primary correlation are significant at R, L, W

and A ratios because of specific processes in teeth in cases of pathological attrition. By regression analysis new formulas were developed (Table 3) and the levels of absolute and relative errors were compared (Table 4).

Discussion

The results depend on the stage of pathological attrition. In the research we have found out that the attrition is caused by bruxism, abnormal occlusion due to dispositioned tooth, and inadequate prosthetic treatment may cause proportional constant intense deposition of tertiary reparative dentine and lowering of occlusal surface depending on pathology stage [12]. However, due to abnormal tooth structures or abrasion factors pathological attrition is not a progressive process during which pulp structures and hard tissues changes depend on time and stage of changes and enhanced process development may take place any time. Also, the better result were established when the mean levels of all six teeth were calculated, and the most distant result were reached when single measurements of mandible canine were included. Improved regressive formulas were checked by new randomized samples of 50 X-ray photos of patients with pathological attrition, no information about age was available. The results were ranged by the error not higher than 14±0.8 years old.

The further research should be focused on verifying value of tertiary reparative dentine using computer cone beam tomography to determine the dynamics of pulp changes intensity in different stages of disorders that cause pathological attrition. It allows making retrospective analysis, which provides information about average changes of pulp chamber and hard tissues of tooth, so identification of physiological secondary dentine formation before the pathology come about and tertiary reparative dentine formation in pathology development gives a chance to create new regression analysis by two systems coefficients 'before pathology' and 'during pathology development'. Thus all minimal errors occur in techniques of age determination in cases of dental health disorders.

Conclusions

During the study we used Kvaal et al. age estimation technique for patients with pathological attrition and defined the level of errors which reaches about 47-49%. Using component regression analysis and Pearson's coefficients we determined the correlation between age results and level of tooth surface attrition and deposition of tertiary reparative dentine due to the kind of pathology, which cause pathological attrition, and the time of pathology. The most significant correlation was found between changes in incisors and lower first premolar. The changes in canine in cases of pathology attrition do not affect the result significantly. Individually calculated modified coefficients in equations by Kvaal et al. age estimation technique in cases of pathological attrition showed the result more adjacent to real age, e.g. the level of absolute error in years was improved from 27±8.4 to 13±0.8 years.

Age estimation technique can be improved taking into account morphological changes in cases of pathological attrition, and the calculated coefficients allow expanding the circle of person's age that should be defined. Also the examination of two regression systems which stand for attrition of occlusal surface and tertiary reparative dentine deposition in cases of pathology (system 1) and lowering of physiological lever of tooth high as well as deposition of secondary dentine (system 2) exclude the number of errors for accurate age estimation with cone beam tomography.

Table 4. Example of differences of age estimated by means of primary technique of Kvaal et al. and modified technique for patient A with pathological attrition

Teeth/tooth groups	Age	Primary technique of Kvaal et al. for patients with pathological attrition		Modified technique of Kvaal et al. for patients with pathological attrition	
		Mean	Difference	Mean	Difference
Single tooth	Actual age	35	21	35	13
	Estimated age	56		48	
All six teeth	Actual age	35	16	35	10
	Estimated age	51		45	
Three maxillary teeth	Actual age	35	18	35	12
	Estimated age	53		47	
Three mandibular teeth	Actual age	35	17	35	14
	Estimated age	52		39	

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PANRESISTANT SUPERBUGS: ARE WE AT THE EDGE OF A 'MICROBIAL HOLOCAUST'

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Contemporary healthcare has progressed towards world health security through advancements in medication-based and surgical interventions, supported by the success of antimicrobial therapy. The emergence of panresistant infectious diseases is becoming a public health problem worldwide. Panresistance is attributable to a complex interplay of antimicrobial overuse in healthcare facilities due to lack of regulatory commitment in the backdrop of natural mutations in pathogens and rise in immunocompromised hosts. Developing countries are facing the brunt in epidemic proportions due to strained public health infrastructure and limited resource allocation to healthcare. Panresistance is a biological, behavioural, technical, economic, regulatory and educational problem of global concern and combating it will require concerted efforts to preserve the efficacy of the available antimicrobials. An intensified commitment needs to be taken up on a war footing to increase awareness in the society, increase laboratory capacity, facilitate antimicrobial research, foster emphasis on infection control and antimicrobial stewardship, and legislation on manufacturing, marketing and dispensing of antimicrobials.

KEY WORDS: **Panresistance; Antimicrobial Resistance; Totally Drug Resistant Tuberculosis; Infection Control; Antimicrobial Stewardship.**

Introduction

Infectious diseases of the antiquity such as plague, cholera, influenza, smallpox, measles and malaria, which have been responsible for claiming billions of lives, have either been eradicated, eliminated or controlled in various parts of the globe due to advanced antimicrobial therapeutics and vaccines. The discovery of penicillin in 1940s and consequent success at wound healing and survival of soldiers in World War II was a major breakthrough in the history of mankind. The subsequent discovery of a series of antimicrobials, some 22 of them credited to Selman Waksman, brought the menace of infectious diseases and ensuing sepsis under control. Antimicrobials conferred safety and reliability upon a wide variety of diagnostic and therapeutic procedures including advanced surgeries, organ transplantation and immunotherapy, being heavily dependent on antimicrobial support.

While the era of infectious diseases was being considered over, backed upon the success of antimicrobial therapy, there was re-emergence of infectious diseases due to rise in immunocompromised populace. The resurgence of infectious diseases consequent to Human Immunodeficiency Virus (HIV)-Acquired Immune Deficiency Syndrome (AIDS) pandemic resulted in 1.5 fold increase in infectious diseases mortality between 1980 and 1992. HIV-AIDS became the leading cause of mortality amongst infectious diseases in the following two decades [1]. Antimicrobial resistance, being unanticipated in its entirety, evolved manifolds to reach dangerous connotations towards panresistance. World Health Organization (WHO) theme for World Health Day 2011 was

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"Antimicrobial resistance: No action today, no cure tomorrow" [2]. Since then, despite progressive steps towards concept development, the magnitude of panresistance overshadows control efforts [3, 4]. Panresistance is a complete roadblock to years of progress made towards advanced healthcare. With infectious diseases being the second leading cause of mortality worldwide as per global health estimates, the day may not be far when it will be the leading cause of death due to emergence of panresistance emanating the realization of a 'Microbial Holocaust'.

Evolution of panresistance

The rise of panresistance is multifactorial. Microbial factors include natural evolution of microorganisms conferring increase in virulence, infectivity, pathogenicity and antimicrobial resistance. Opportunistic pathogens are crossing host barriers and are now being encountered as emerging pathogens [1, 5, 6, 7]. Established pathogens are evolving into panresistant potentially untreatable mutants such as glycopeptide resistant Gram negative bacteria, which are resistant to all available antimicrobials including tigecycline and colistin. In addition, totally drug resistant tuberculosis, multidrug resistant malaria and dual oseltamivir-adamantane resistant influenza viruses are emerging [8]. Tuberculosis has emerged as the leading cause of mortality amongst infectious diseases overtaking HIV-AIDS due to the development of resistance. Tuberculosis related deaths in 2014 were 1.5 million, surpassing 1.2 million HIV-AIDS related deaths.

Host factors include steep rise in immunocompromised populace owing to increased organ transplants, immunodeficiency disorders, neoplasms, old age as well as patients under intensive-care. Prescription trend factors include aggressive exposure of multiple antimicrobials to patients harbouring multiresistant microorganisms. Rising empiricism in antimicrobial therapy overshadows susceptibility guided therapy, facilitating development of resistance due to selection pressure [9, 10]. Panresistant microorganisms can spread resistance-conferring mobile genetic elements to susceptible microorganisms and commensal flora, contributing to the development of a reservoir of antimicrobial resistance in human body. Panresistant microorganisms can also colonize inanimate surfaces and create reservoirs from which they can get transmitted in healthcare facilities thereby rendering all patients and healthcare professionals at-risk. Human factors involved include a complex interplay of antimicrobial misuse in healthcare facilities due to lack of regulatory commitment in the backdrop of natural mutations which has contributed to the development of panresistance [1, 3]. Panresistance is increasingly being reported in Gram negative microbes [6, 11]. The South and South-East Asia region (SEAR) has one of the highest prevalence of tuberculosis with one death every few minutes [12]. All forms of resistant tuberculosis viz. Multi drug resistant tuberculosis (MDR TB), extremely drug resistant tuberculosis (XDR TB) and totally drug resistant tuberculosis (TDR TB) have been reported. The recent reports of TDR TB from Iran, India and Italy represent the tip of an iceberg as antitubercular susceptibility testing occurs in only 5% patients worldwide [12-15]. The DOTS (Directly Observed Treatment Short course) program for developing countries has been challenged by the emergence of XDR TB and TDR TB not only due to resistance but also due to limitations of antitubercular susceptibility testing, which is offered only at highly specialized centres. A seven year study on DOTS plus reported 61% cure, 19% deaths, 18% defaulters, 3% failed treatments and an average delay of 5 months in initiation of therapy [16]. Antimalarial resistance to artemisinin and quinine has been reported in SEAR and Africa [17, 18]. Antiviral resistance to almost all antivirals has been reported particularly in Hepatitis B, Herpes virus, Cytomegalovirus, Varicella zoster, Influenza and HIV [19–23].

Impact of panresistance

The emergence of panresistant infectious diseases is becoming a public health problem worldwide. Developing countries are facing the brunt in epidemic proportions due to strained public health infrastructure and limited resource allocation to healthcare. The rise of panresistance is discouraging the development of newer antimicrobials under private equity. Any new antimicrobial loses economic value in a few years due to emergence of resistance compared to medicines for lifestyle diseases which remain economically rewarding for many years [24, 25]. Inadvertent or intended release of panresistant bioweapons against humans, fauna and flora can wreak havoc leading to widespread disruption [26].

The future

Panresistance is a biological, behavioural, technical, economic, regulatory and educational problem of global concern and combating it will require concerted efforts to preserve the

efficacy of available antimicrobials. An intensified commitment needs to be taken up on a war footing.

Knowledge, Attitude and Practices of General Public

Examples from successful programs such as "Antibiotics are not Automatic" in France, "Get Smart" in the US and "Do Bugs Need Drugs?" in Canada need to be followed in developing countries [27]. Health educators, public health specialists, government officials and community leaders should be sensitized about the hazards of using antimicrobials. There should be active community participation in the cause of positive health. Citizens must foster sound belief and inculcate positive attitude and responsible behaviour in societal healthcare system. There is long standing need for increasing awareness about approach, operation, decision making and scope of healthcare amongst general population. Attitude and expectations need a paradigm shift from 'instant cure' and 'magic pill' to 'rational drug therapy' and 'evidence based healthcare'. Patients should not engage into unjustified requests or arguments or frequent change of doctor's advice. Self-medication, quack remedies, underdosing and uncompleted regimens should be stopped. Left over drugs from the last prescription should not be taken again for a similarly perceived symptom. The society should ensure availability of trained pharmacists through legislation to ensure adherence to prescription safety.

Hospital Infection Control

Nosocomial pathogens evolve under continuous selection pressure to become panresistant. Hospital Infection Control involves monitoring of hospital safety measures such as patient isolation, visitor control, contact precautions, barrier nursing, universal prophylaxis, hand hygiene, environmental surveillance and equipment sterilization in operation theatre, labour room, intensive care, oncology, burns, dialysis and transplant centres. Carriers are identified, quarantined and organisms are eradicated from hospital environment. Hand hygiene is considered to be the single most important step in controlling spread of panresistant pathogens. Compliance is limited due to overbearing pressures of patient volume, time, undue multitasking and paucity of washing infrastructure. Hand washing with soap followed by antiseptic handrub should be strongly encouraged as the standard of care for all healthcare practitioners and patients. A broad based policy and standards for infection control in healthcare facilities needs to be implemented. The Jaipur declaration on AMR-2011 for SEAR and the Chennai declaration for India are efforts to this end [25, 28].

Laboratory Surveillance

Laboratory based surveillance of infectious diseases, pathogens, susceptibility patterns, resistance phenotyping, outbreak investigation, hospital environmental surveillance and epidemiological typing is mandated to keep a track of panresistance development. A number of pathogens such as viruses, parasites, certain bacteria and fungi surpass identification under the constraints of resources available in routine labs [1, 5–7]. Antimicrobial susceptibility testing for tuberculosis, parasites, viruses and fungi are only available in reference labs which are far and few. Unavailability of testing facilities promotes empirical antimicrobial therapy to save the patient, thereby contributing to the development of panresistance. Enhancing laboratory capacity with automated phenotypic identification systems, molecular microbiology techniques and biostatistical softwares, precise organism identification to species level, antimicrobial susceptibility patterns, resistance phenotypes, typing and data analysis has been facilitated. The resistogram generated can be used to guide infection control strategies with other collaborating centres through a worldwide free web repository. The potential of the microbiology lab is largely underutilized in developing countries due to deficiencies in lab equipment and specialized staff.

Antimicrobial Stewardship

Antimicrobial stewardship including antimicrobial rotation and holiday, combination therapy and Standard Treatment Guidelines have proven to be beneficial [29, 30]. A dynamic antimicrobial policy should specify as to when escalation and de-escalation to reserve antimicrobials such as carbapenems, colistin, tigecycline, vancomycin, teicoplanin and daptomycin, needs to be undertaken. Spiralling empiricism, prophylactic antimicrobial usage and attitude to use the best antimicrobial should be discouraged and susceptibility guided therapy be promulgated [1, 5–7]. Regular availability of required antimicrobials, prescription audits, formulary restriction, pre-authorization and stop orders should be advocated to ensure policy implementation which in turn should be linked to grant of accreditation to hospitals. A multidisciplinary approach would include building of consensus across

clinicians and arbitration of disagreements. The WHO classification of antimicrobials into key, watch and reserve groups in Jun 2017 can form a guideline towards the successful implementation of antimicrobial authorization and prescription prudence [31].

Health Resource Allocation

The present situation demands an increase in resource allocation in the health sector to boost healthcare infrastructure, public awareness and accessibility. This would entail accommodative policy for establishment of specialized medical varsities, superspeciality hospitals, specialized laboratories, biocontainment facilities, promotion of antimicrobial research through grants, medical journals, medical societies, involvement of private sector through public private partnership and mass health campaigns. Comprehensive standards for surveillance and control should be established in association with international health regulations [32]. National surveillance systems similar to the National Nosocomial Infection Surveillance (NNIS) in the US and SENTRY Antimicrobial Surveillance Program can be instituted [32].

Antimicrobial Research

Research on the development of newer antimicrobials has multipronged implications. One, effective antimicrobials would foster prompt treatment of infections caused by resistant pathogens and prevent progression to disseminated infection and sepsis. Two, successful therapy will reduce transmission of resistant pathogens. Three, chemoprophylaxis can be directed for prevention of infections in susceptible host population. Four, behavioural research regarding non-adherence to prescribed drug schedules and self-medication are social issues in which there has been limited research. Research needs to be undertaken on these behavioural factors, so that targeted intervention can be planned for bringing about changes at the societal level.

Public Health Measures

Robust public health infrastructure and human resource with strengthened vector control programs, immunization coverage, screening programs, national health programs, rapid outbreak investigation, quarantine and control measures are required. Panresistant infectious diseases and resistant pathogens should be made notifiable. Effective public health will reduce reliance on antimicrobials and break chain of transmission of resistant microbes [25].

Legislation on Manufacturing, Marketing and Dispensing of Antimicrobials

Three important areas of intervention exist at manufacturing, marketing and dispensing of antimicrobials. Quality assurance in antimicrobial dosage and efficacy from manufacturers and ethical marketing can have profound downstream effects. While regulation regarding prohibition of sale of antimicrobials without proper prescriptions is in place, it is not being implemented. Regulatory mechanisms for ensuring good manufacturing practices, responsible marketing and dispensation by pharmacists need to be instituted and strengthened through industrial and marketing audit, and enhanced vigil on pharmacies. Antimicrobial and infection control advisory bodies need to be actively involved to integrate surveillance and legislation.

Role of WHO

WHO has issued a call for action to halt the spread of AMR by introducing a six-point policy package for all countries to combat AMR. This includes commitment to a comprehensive, financed national plan with accountability and civil society engagement; strengthening of surveillance and laboratory capacity; ensuring uninterrupted access to essential medicines of assured quality; regulation and promotion of rational use of medicines, including in animal husbandry, and ensuring proper patient care; reduction of antimicrobials usage in food-producing animals; enhancing infection prevention and control; and fosterage of innovations and research and development for new tools [2]. WHO has also laid down the procedure to establish national laboratory based surveillance including identification of pathogens and diseases of public health importance, creation of network of Antimicrobial Susceptibility Testing (AST) and standardization of involved methodologies. World bodies such as Association for Prudent Use of Antimicrobials (APUA) and World Alliance against Antibiotic Resistance (WAAR) are efforts to this end [33]. WHO has advocated a priority pathogens list in 2017 to highlight a list of bacteria for which newer antimicrobials are urgently required [34]. WHO has classified antimicrobials into key access, watch group and reserve group to optimize usage guidelines worldwide [35].

Conclusions

Panresistance is emerging in alarming proportions worldwide, thereby threatening the advances made towards public health

security of the world. There is a dire need to identify this threat, develop concerted multipronged strategy, develop infrastructure, foster expertise and take coordinated and urgent steps to tackle the serious public health challenge. It is time for action else we face the consequences of microbial genocide of mankind. Resolute conviction towards astute measures with a sustained momentum will hold a promise for safeguarding health of future generations.

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ACETAMINOPHEN EFFECT ON FREE RADICAL OXIDATION INDICES IN RATS WITH TYPE 2 DIABETES MELLITUS

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Background. *Acetaminophen is a drug used to relieve pain syndrome. It is used both independently and in composition of combined drugs. Type 2 diabetes is an age-related disease that is associated with a violation of insulin synthesis by pancreas.*

Objective. *The aim of the research was to study the effect of acetaminophen on major free radical oxidation indices of rats with type 2 diabetes mellitus in time dynamics.*

Methods. *We conducted two series of experiments. The first series comprised rats with type 2 diabetes mellitus and acute acetaminophen toxic lesions. The second series involved rats with type 2 diabetes mellitus and acetaminophen administration at a dose of 55 mg/kg for the period of 7 days.*

Results. *Administration of acetaminophen for rats with type 2 diabetes mellitus caused the increase in the content of malondialdehyde, diene and triene conjugates and Schiff bases in blood plasma and malondialdehyde, diene and triene conjugates in liver homogenate. The maximum increase in these indices was observed on the first day of the experiment. Gradually these indices decreased on the 3rd, 5th and 7th days of the experiment.*

Conclusions. *Free radical oxidation increased in both series of the experiment. This process developed in rats with type 2 diabetes mellitus and acute acetaminophen toxic lesions more intensively, than in rats with type 2 diabetes mellitus and administration of acetaminophen at the highest therapeutic dose during 7 days.*

KEY WORDS: **acetaminophen; malondialdehyde; diene and triene conjugates; Schiff bases; diabetes mellitus.**

Introduction

Acetaminophen is a drug used to relieve pain syndrome. It is used both independently and in composition of combined drugs. A long time it was considered to be the safest drug among the group of analgesics/antipyretics [1, 2]. Acetaminophen has a relatively low toxicity in therapeutic doses. However, a conscious and often uncontrolled administration of high doses of the drug causes complications that sometimes can lead to death due to hepatic insufficiency [3, 4, 5].

Type 2 diabetes is one of the most common diseases, every year its frequency is steadily increasing. The prevalence of diabetes is associated with changes in environmental factors, especially the populations (genetic, demographic), the concentration of risk factors in the populations (increased body weight, arterial hypertension, cardiovascular diseases, lipid metabolism disorders, etc.).

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Considering all above, the aim of our research was to study free radical oxidation activity in rats with type 2 diabetes mellitus and acetaminophen toxic lesions.

Methods

The experiments were carried out on white rats weighing 180–220 g on a standard diet and free access to water in vivarium.

We conducted two series of experiments. In the first series toxic lesion was caused by a single intragastric administration of acetaminophen suspension in 2% starch solution for the animals at a dose of 1250 mg/kg (1/2 LD_{50}). In the second series the suspension of acetaminophen in a 2% starch solution at a dose of 55 mg/kg was managed, which corresponds to the highest therapeutic dose during 7 days. Non-genetic form of experimental type 2 diabetes mellitus was modeled by Islam S., Choi H. method [7, 8], that is, a single intraperitoneal administration of streptozotocin solution at a dose of 65 mg/kg (Sigma, USA) to the rats, which was diluted by citrate buffer (pH 4.5) with the previous (15 minutes ahead) intraperitoneal nicotinamide

administration at a dose of 230 mg/kg. The rats with the same body weight, which were given the same amount of solvent (citrate buffer pH 4.5), were used as the control group.

In the first series of the experiment, the rats were divided into 4 groups: the 1st group was the intact (control); the 2nd group involved a single acetaminophen administration; the 3rd group comprised the animals with type 2 diabetes mellitus caused by streptozotocin administration; and the 4th group contained the rats with a single administration of acetaminophen after streptozotocin administration. In the second series of the experiment, the rats were divided into 4 groups: the 1st group was the intact (control); the 2nd group was with acetaminophen administration during 7 days; the 3rd group were the animals with type 2 diabetes mellitus caused by streptozotocin administration; the 4th group were the rats with administration of acetaminophen during 7 days after streptozotocin administration.

The animals were removed from the experiment on the 5th, 3rd, 5th and 7th days after last acetaminophen administration by euthanasia under thiopental anaesthesia. All experiments on rats were carried out according to The Guideline Principles for the Care and Use of Laboratory Animals [10].

Evaluation of the content of diene and triene conjugates was carried out by the method [6]. Determination of malondialdehyde content was carried out by the method [9]. Determination of the contents of the Schiff bases was carried

Table 1. Dynamics of content of malondialdehyde, diene and triene conjugates and Schiff bases in blood plasma of rats with type 2 diabetes mellitus and acute acetaminophen toxic lesions (M±m; n=10)

Group of animals	Time after acetaminophen administration (days)				
	Content of	1st day	3rd day	5th day	7th day
Control n=10	MDA, μmol/l	7.48±0.47			
	DC, U/l	1.12±0.03			
	TC, U/l	0.58±0.01			
	Schiff bases, U/l	2.53±0.33			
Acetaminophen (single) n=10	MDA, μmol/l	24.62±1.42 p_1<0.001	22.63±1.11 p_1<0.001	21.79±1.12 p_1<0.001	21.47±1.13 p_1<0.001
	DC, U/l	4.62±0.49 p_1<0.001	4.47±0.41 p_1<0.001	3.42±0.48 p_1<0.001	3.11±0.47 p_1<0.001
	TC, U/l	1.97±0.37 p_1<0.001	1.80±0.24 p_1<0.001	1.76±0.32 p_1<0.001	1.65±0.31 p_1<0.001
	Schiff bases, U/l	8.61±0.77 p_1<0.001	8.04±0.71 p_1<0.001	7.79±0.65 p_1<0.001	7.60±0.62 p_1<0.001
Type 2 DM n=10	MDA, μmol/l	14.62±0.92 p_1<0.001	14.22±1.02 p_1<0.001	14.03±0.69 p_1<0.001	13.56±0.65 p_1<0.001
	DC, U/l	1.831±0.24 p_1<0.001	1.79±0.31 p_1<0.001	1.72±0.28 p_1<0.001	1.66±0.31 p_1<0.001
	TC, U/l	1.20±0.18 p_1<0.001	1.17±0.23 p_1<0.001	1.08±0.16 p_1<0.001	0.95±0.18 p_1<0.001
	Schiff bases, U/l	5.10±0.47 p_1<0.001	5.01±0.59 p_1<0.001	4.87±0.44 p_1<0.001	4.63±0.52 p_1<0.001
Acetaminophen (rats with Type 2 DM) n=10	MDA, μmol/l	39.01±1.12 p_1<0.001 p_2<0.001	38.72±0.91 p_1<0.001 p_2<0.001	38.13±1.19 p_1<0.001 p_2<0.001	37.58±1.22 p_1<0.001 p_2<0.001
	DC, U/l	3.86±0.28 p_1<0.001 p_2<0.001	3.70±0.50 p_1<0.001 p_2<0.001	3.38±0.51 p_1<0.001 p_2<0.001	3.14±0.52 p_1<0.001 p_2<0.001
	TC, U/l	4.00±0.39 p_1<0.001 p_2<0.001	3.91±0.49 p_1<0.001 p_2<0.001	3.77±0.54 p_1<0.001 p_2<0.001	3.53±0.48 p_1<0.001 p_2<0.001
	Schiff bases, U/l	13.33±1.15 p_1<0.001 p_2<0.001	13.20±0.93 p_1<0.001 p_2<0.001	12.98±0.97 p_1<0.001 p_2<0.001	12.81±0.61 p_1<0.001 p_2<0.001

Notes: here and in the following tables
p_1 – significant difference compare with control animals;
p_2 – significant difference compare with the animals, which were administered with acetaminophen.

out by the method [12, 13]. Quantitative indices were processed statistically. The results of the experiment were processed by means of statistical program Statistica [11] using parametric Student's t test and Wilcoxon signed-rank test for non-parametric statistical hypothesis test. Changes were considered significant at $p \leq 0.05$.

Results

The content of malondialdehyde, diene and triene conjugates and Schiff bases increased in animals with lesions caused by acetaminophen and type 2 diabetes mellitus.

As presented in Table 1, the content of malondialdehyde in blood plasma increased by 229.2% on the 1st day of the experiment in the 2nd group of experimental animals, it increased by 95.5% in the 3rd group of animals. The maximum of this index increase (in 5.21 times) was observed in the animals with type 2 diabetes mellitus and acute acetaminophen toxic lesions (the 4th group). This index decreased on the 3rd and 5th days of the experiment. The maximal decrease of malondialdehyde content was observed on the 7th day of the experiment in all groups of animals.

The content of diene and triene conjugates in blood plasma of the animals with single acetaminophen administration (the 2nd group) increased in 4.11 and 3.36 times on the 1st day of the experiment to compare with the control animals. These indices increased by 62.8% and 104.8% in the 3rd group of animals with streptozotocin action. The content of diene conjugates increased in 3.43 times, and triene conjugates in 6.81 times in the animals with type 2 diabetes mellitus and acute acetaminophen toxic lesions (the 4th group). These indices decreased on the 3rd, 5th and 7th days of the experiment.

The content of Schiff bases increased in 3.4 times on the 1st day of the experiment in the 2nd group of experimental animals after acetaminophen administration. This index increased by 101.6% in the 3rd group of animals with streptozotocin administration. The maximal increase of Schiff bases in 5.26 times was observed in the 4th group of animals with type 2 diabetes mellitus and single acetaminophen administration.

According to the results (Table 2), the content of malondialdehyde in liver homogenate increased in 4.76 times on the 1st day of the experiment in the 2nd group of experimental animals to compare with the control animals, and by 60.8% in the 3rd group of animals. The maximum of this index increase (in 5.7 times) was observed in the 4th group of animals.

Table 2. Dynamics of content of malondialdehyde, diene and triene conjugates in liver homogenate of rats with type 2 diabetes mellitus and acute acetaminophen toxic lesions (M±m; n=10)

Group of animals	Time after acetaminophen administration (days)				
	Content of	1st day	3rd day	5th day	7th day
Control n=10	MDA, μmol/l	13.67±1.16			
	DC, U/l	7.83±0.26			
	TC, U/l	3.69±0.16			
Acetaminophen (single) n=10	MDA, μmol/l	65.10±3.57 $p_1<0.001$	64.51±3.50 $p_1<0.001$	63.67±2.41 $p_1<0.001$	62.63±2.11 $p_1<0.001$
	DC, U/l	46.09±1.43 $p_1<0.001$	44.21±2.07 $p_1<0.001$	39.47±1.72 $p_1<0.001$	38.94±2.06 $p_1<0.001$
	TC, U/l	17.93±0.78 $p_1<0.001$	17.18±1.56 $p_1<0.001$	16.14±1.69 $p_1<0.001$	15.38±1.28 $p_1<0.001$
Type 2 DM n=10	MDA, μmol/l	21.98±1.80 $p_1<0.001$	21.55±2.04 $p_1<0.001$	20.79±1.92 $p_1<0.001$	20.19±2.08 $p_1<0.001$
	DC, U/l	23.44±1.00 $p_1<0.001$	21.66±1.50 $p_1<0.001$	20.47±1.13 $p_1<0.001$	20.19±1.70 $p_1<0.001$
	TC, U/l	13.43±0.58 $p_1<0.001$	12.78±1.37 $p_1<0.001$	12.02±1.04 $p_1<0.001$	11.47±1.11 $p_1<0.001$
Acetaminophen (rats with type 2 DM) n=10	MDA, μmol/l	77.94±2.43 $p_1<0.001$ $p_2<0.001$	77.31±4.14 $p_1<0.001$ $p_2<0.001$	76.53±2.00 $p_1<0.001$ $p_2<0.001$	75.70±2.09 $p_1<0.001$ $p_2<0.001$
	DC, U/l	52.98±1.03 $p_1<0.001$ $p_2<0.001$	51.25±1.89 $p_1<0.001$ $p_2<0.001$	51.03±2.27 $p_1<0.001$ $p_2<0.001$	47.34±2.34 $p_1<0.001$ $p_2<0.001$
	TC, U/l	24.24±1.49 $p_1<0.001$ $p_2<0.001$	23.56±1.45 $p_1<0.001$ $p_2<0.001$	22.94±1.67 $p_1<0.001$ $p_2<0.001$	22.01±1.64 $p_1<0.001$ $p_2<0.001$

The content of diene and triene conjugates in the experimental animals increased the most in the 4th group of animals on the 1st day of the experiment in 6.76 and 6.56 times respectively. These indices increased on the 1st day of the experiment in 5.88 and 4.85 times in the 2nd group, and in 2.99 and 3.63 times in the 3rd group respectively.

The content of malondialdehyde, diene and triene conjugates, Schiff bases increased on the 1st day of the experiment in blood plasma (Table 3) and liver homogenates (Table 4). These indices decreased on the 3rd, 5th, 7th days in all series of the experiment. These changes were less pronounced than in the 1st series of the experiment.

In the 2nd group of experimental animals with acetaminophen administration during 7 days, the content of malondialdehyde in blood plasma on the 1st day of the experiment increased by 126.5%, in liver homogenate – by 138.6%; diene and triene conjugates in blood plasma increased by 105.9% and 142.3%, in liver homogenate increased in 3.0 and 3.2 times; Schiff bases increased by 134.3% in blood plasma to compare with control animals.

In animals with type 2 diabetes and acetaminophen administration during 7 days (the 4th group), malondialdehyde in blood plasma increased the most in 4.13 times on the 1st day of the experiment, it increased in 4.9 times in lever homogenate; the content of diene and triene conjugates increased in blood plasma in 3.1 and 3.2 times, in 5.7 and 4.2 times in liver homogenate; Schiff bases increased in 4.1 times in blood plasma.

Discussion

The activation of free radical processes is universal mechanism in case of toxic action of the vast majority of toxic agents. The mechanism of cell damage by free radical metabolites,

Table 3. Dynamics of content of malondialdehyde, diene and triene conjugates and Schiff bases in blood plasma of rats with type 2 diabetes mellitus and acetaminophen administration at a dose of 55 mg/kg during 7 days (M±m; n=10)

Group of animals	Time after acetaminophen administration (days)				
	Content of	1st day	3rd day	5th day	7th day
Control n=10	MDA, μmol/l	7.48±0.47			
	DC, U/l	1.12±0.03			
	TC, U/l	0.58±0.01			
	Schiff bases, U/l	2.53±0.33			
Acetaminophen (7 days) n=10	MDA, μmol/l	16.94±0.94 p_1<0.001	16.54±0.99 p_1<0.001	16.12±1.04 p_1<0.001	15.77±0.96 p_1<0.001
	DC, U/l	2.31±0.29 p_1<0.001	2.24±0.20 p_1<0.001	2.00±0.21 p_1<0.001	1.83±0.21 p_1<0.001
	TC, U/l	1.42±0.07 p_1<0.001	1.24±0.21 p_1<0.001	1.19±0.20 p_1<0.001	1.17±0.28 p_1<0.001
	Schiff bases, U/l	5.93±0.64 p_1<0.001	5.75±0.71 p_1<0.001	5.58±0.77 p_1<0.001	5.43±0.65 p_1<0.001
Type 2 DM n=10	MDA, μmol/l	14.62±0.92 p_1<0.001	14.22±1.02 p_1<0.001	14.03±0.69 p_1<0.001	13.56±0.65 p_1<0.001
	DC, U/l	1.831±0.24 p_1<0.001	1.79±0.31 p_1<0.001	1.72±0.28 p_1<0.001	1.66±0.31 p_1<0.001
	TC, U/l	1.20±0.18 p_1<0.001	1.17±0.23 p_1<0.001	1.08±0.16 p_1<0.001	0.95±0.18 p_1<0.001
	Schiff bases, U/l	5.10 ± 0.47 p_1<0.001	5.01 ± 0.59 p_1<0.001	4.87 ± 0.44 p_1<0.001	4.63 ± 0.52 p_1<0.001
Acetaminophen (rats with Type 2 DM) n=10	MDA, μmol/l	30.90±0.55 p_1<0.001 p_2<0.001	30.54±0.73 p_1<0.001 p_2<0.001	29.91±0.87 p_1<0.001 p_2<0.001	29.40±1.31 p_1<0.001 p_2<0.001
	DC, U/l	3.52±0.21 p_1<0.001 p_2<0.001	3.25±0.45 p_1<0.001 p_2<0.001	2.85±0.37 p_1<0.001 p_2<0.001	2.46±0.39 p_1<0.001 p_2<0.001
	TC, U/l	1.88±0.33 p_1<0.001 p_2<0.001	1.83±0.33 p_1<0.001 p_2<0.001	1.76±0.36 p_1<0.001 p_2<0.001	1.63±0.30 p_1<0.001 p_2<0.001
	Schiff bases, U/l	10.60±0.77 p_1<0.001 p_2<0.001	10.41±0.82 p_1<0.001 p_2<0.001	10.22±0.82 p_1<0.001 p_2<0.001	10.03±0.68 p_1<0.001 p_2<0.001

Table 4. Dynamics of content of malondialdehyde, diene and triene conjugates and Schiff bases in liver homogenate of rats with type 2 diabetes mellitus and acetaminophen administration at a dose of 55 mg/kg during 7 days (M±m; n=10)

Group of animals	Time after acetaminophen administration (days)				
	Content of	1st day	3rd day	5th day	7th day
Control n=10	MDA, μmol/l	13.67±1.16			
	DC, U/l	7.83±0.26			
	TC, U/l	3.69±0.16			
Acetaminophen (7 days) n=10	MDA, μmol/l	32.62±1.75 p_1<0.001	31.83±2.78 p_1<0.001	31.34±1.63 p_1<0.001	29.79±1.84 p_1<0.001
	DC, U/l	23.63±1.60 p_1<0.001	23.45±1.42 p_1<0.001	21.43±1.40 p_1<0.001	20.82±1.74 p_1<0.001
	TC, U/l	12.02±0.79 p_1<0.001	11.22±0.90 p_1<0.001	10.91±0.99 p_1<0.001	10.03±0.90 p_1<0.001
Type 2 DM n=10	MDA, μmol/l	21.98±1.80 p_1<0.001	21.55±2.04 p_1<0.001	20.79±1.92 p_1<0.001	20.19±2.08 p_1<0.001
	DC, U/l	23.44±1.00 p_1<0.001	21.66±1.50 p_1<0.001	20.47±1.13 p_1<0.001	20.19±1.70 p_1<0.001
	TC, U/l	13.43±0.58 p_1<0.001	12.78±1.37 p_1<0.001	12.02±1.04 p_1<0.001	11.47±1.11 p_1<0.001
Acetaminophen (rats with Type 2 DM) n=10	MDA, μmol/l	67.00±1.78 p_1<0.001 p_2<0.001	66.54±2.63 p_1<0.001 p_2<0.001	65.66±2.20 p_1<0.001 p_2<0.001	65.43±2.18 p_1<0.001 p_2<0.001
	DC, U/l	44.75±1.14 p_1<0.001 p_2<0.001	42.88±1.55 p_1<0.001 p_2<0.001	41.51±1.67 p_1<0.001 p_2<0.001	39.14±1.70 p_1<0.001 p_2<0.001
	TC, U/l	15.62±1.12 p_1<0.001 p_2<0.001	15.19±1.26 p_1<0.001 p_2<0.001	14.92±1.25 p_1<0.001 p_2<0.001	14.42±1.25 p_1<0.001 p_2<0.001

which are formed as a result of large number of biocidal xenobiotics biotransformation, including acetaminophen, involves their ability to initiate processes of lipid peroxidation and oxidation modification of proteins, covalently bind with bio-macromolecules (proteins, nucleic acids, lipids) and generate reactive oxygen intermediates (ROI), which are highly toxic and capable to initiate new chains of free radical reactions. The severity of damage effect of free radicals depends on the intensity of their production and functional ability of antioxidant system. As the result of metabolic transformations of acetaminophen, as proved by a number of researchers [1, 2], free radical metabolites, as well as reactive oxygen intermediates are formed; the damage by acetaminophen is accompanied by the intensification of free radical processes. Detoxification system is exhausted due to hyperglycaemia induced by streptozotocin injections, and reactive metabolites of acetaminophen prove even more toxic damage, the intensification of reactive oxygen intermediates formation and activation of lipid peroxidation processes in particular. Consequently, we can assert that the radical oxidation processes in the rats with type 2 diabetes and acetaminophen action increases significantly that may cause excessive formation of free radicals and violation of their neutralization.

Conclusions

Acute lesions by acetaminophen (1/2 LD_{50}) of animals with type 2 diabetes causes significant increase of lipids peroxidation processes in comparison with the animals without the simulated pathological process, and non-*diabetic* animals, which were modeled by acetaminophen poisoning, as indicated by the increase in the concentration of diene and triene conjugates, TBA-active products, and Schiff bases.

The administration of acetaminophen at a higher therapeutic dose for the rats with hyperglycemia during 7 days was also accompanied by a significant increase in the concentration of lipid peroxidation products, but less pronounced than in cases of acute lesions.

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ECOLOGICAL FEATURES OF MICROBIOCENOSIS OF THE SKIN OF MAMMARY GLANDS AND VAGINA IN PREGNANT WOMEN WITH THREAT OF PRETERM LABOR

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Background. *The threat of preterm birth is one of the most topical issues in the world medicine. According to the statistics, from 12–13 to 25–35 % of all pregnancies end prematurely. One of the causes of preterm labour is chronic inflammatory processes of female genital organs and disorder of microbiocenosis. Timely diagnosis and adequate treatment will reduce the risk of premature labour and avoid perinatal loss.*

Objective. *We examined and analysed the microflora of the skin of mammary glands and mucous membrane of vagina in healthy pregnant women and patients with threat of preterm labour.*

Methods. *The examination of the pregnant was conducted at the TRMPC "Mother and Child" in several stages. First of all, we rinsed the skin of mammary glands and smeared from mucous membrane the posterior vault of vagina with sterile swabs pre-moistened in physiological solution. After that, the tampons were placed in sterile tubes and delivered to laboratory. Sowing was carried out on Petri dishes with sterile medium: ZHSA, bloods MPA, Endo, Saburo, thioglycolic medium.*

Results. *As a result of the research we found saprophytic Gram-positive and Gram-negative microorganisms (in women with a physiological course of pregnancy). In pregnant women with preterm labor, there is an increase in the number of St. haemolyticus from 13% to 87%, appearance of representatives of the pathogenic flora – St. aureus (in 20%).*

Conclusions. *Changes in the microbiocenosis of the mammary glands and mucous membranes of the vagina of pregnant women with preterm labor may indicate the presence of opportunistic microflora, or personal hygiene or the presence of associated bacterial infections. It requires the further investigation of possible links among the preterm birth and microbiota.*

KEY WORDS: **microbiocenosis of the skin of mammary glands; mucous membrane of vagina; preterm labour.**

Introduction

Currently, the threat of preterm birth is one of the most pressing issues in the world. According to literary statistics, from 12–13 to 25–35% of all pregnancies ended prematurely [1, 3]. One of the causes of preterm labour is chronic inflammatory processes of female genital organs and disorder (dysbiosis) of microbiocenosis [2]. Timely diagnosis and adequate treatment reduce the risk of premature labour and avoid perinatal loss. In normal conditions, women are dominated by lactic acid bacteria in vagina, which prefer to live in an acidified medium of healthy vagina [4, 6]. They protect mucous membrane and may show slight scaling of mucous membranes. If this Lactobacillus becomes little, conditionally pathogenic microbes begin to multiply instead of them. They are also normally in a womb of a healthy woman, but in very small quantities. If there are many of them the imbalance presents and bacterial vaginosis develops [5].

According to the literature, the main causes of the imbalance of microorganisms are:

– changes of hormonal state in pregnancy, when progestogen hormones shift the vaginal medium to the paralysis;

– dysbiosis in intestine, which promotes changes in the balance of microbes in vagina;

– the use of drugs during pregnancy that affect microflora;

– chronic infectious processes in the body of a pregnant woman with chronic inflammation in the urinary tract that is most often relevant;

– chronic sex infections are not detected in time and aggravated during pregnancy (Ford H. B, Schust D. J, 2009).

Methods

The examination of the pregnant was conducted at Ternopil Regional Municipal Perinatal

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Centre "Mother and Child" in several stages. First of all, we rinsed the skin of mammary glands and smeared from mucous membrane the posterior vault of vagina with sterile swabs pre-moistened in physiological solution. We took the material by scrolling all sides of cotton swab. After that, the tampons were placed in sterile tubes and delivered to laboratory. The time was 20-30 minutes from the stage of taking the research material to the crop. Sowing was carried out on Petri dishes in sterile medium: ZHSA, bloods MPA (for detection of coccious microorganisms), Endo (Enterobacteriaceae), Saburo (mushrooms of genus Candida), thioglycolic medium (anaerobic microorganisms). Each of the cups was marked with the indication accordingly. Sowing on the medium was carried out with tampons: first touching one edge of Petri cup, then – scrolling with all sides. Then we continued sowing the sterilized bacteriological loop perpendicular to the sowing with a swab. The media was placed in a thermostat for 18-48 hours at an optimum temperature. We evaluated the growth of microorganisms on the media after incubation in the thermostat (their shape, colour, size of colonies, nature of surface and edges). Next, we made smears from certain types of colonies, stained with Gram method and microscopically.

Results

As a result of microscopic examination, in 15 women from the control group (pregnant women with physiological pregnancy) were found:

– **on the skin of mammary gland** E. soli, Fusobacterium, Gram-positive non-spore baillus, M. roseus, Streptococcus spp., lactose-negative Enterobacteria in 7%; Lactobacillus, St. haemolyticus – in 13%; St. saprophyticus – in 20%; Corynebacterium – in 27%; Clostridium, Tetracoccus, Bacillus spp. – in 33%; M. luteus – in 40%, Peptostreptococcus, St. epidermidis, M. lylae – in 47%; Bacteroideus – in 60% of the examined patients were present;

– **in the vaginal smears** St. hominis, Streptococcus, Gram-positive non-spore bacillus, St. haemolyticus in 7%; Streptobacillus, Streptococcus spp., M. lylae – in 13%; Tetracoccus, Candida, E. soli, St. saprophyticus, Lactose-negative Enterobacteria – in 20%; M. luteus – in 33%; Doderlein sticks, Corynebacterium, St. epidermis, Bacillus spp. – in 40%; Clostridium, Bacteroideus, Enterococcus – in 47%; Lactobacillus – in 73% of the examined women were evidenced.

In the patients with a threat of preterm labour, it was found:

– **on the skin of mammary glands**: M. sedentarius, St. hominis, M. roseus, Lactobacillus, Gamma-hemolyticus streptococcus – in 7%; M. varians, Bacillus spp. – in 13%; Co-

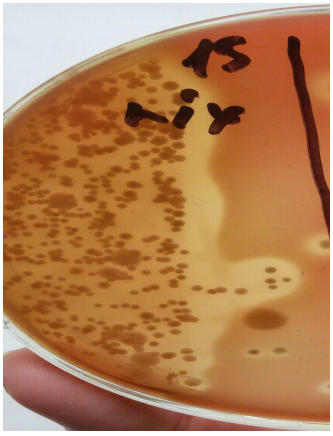

Fig. 2. Cultures of microorganisms on Petri dishes (bloods MPA medium)

Fig. 1. Cultures of microorganisms on Petri dishes (Endo medium)

Fig. 3. Cultures of microorganisms on Petri dishes (ZHSA medium)

rynebakterium spp., St. aureus, M. lylae – in 20%; M. luteus, St. epidermidis – in 33%; St. haemolyticus – in 87% of the exemined;

– **in the vagina smears**: St. epidermidis, M. luteus, Streptobacillus in – 7%; St. aureus, Lactose-negative Enterococcus – in 13%; Alpha-hemolyticus streptococcus, Beta-hemolyticus streptococcus in – 20%; Bacillus spp., Candida, E. coli – in 27%; Enterococcus – in 53%; St. haemolyticus in 67% of the examined pregnant women.

Discussion

According to foreign statistics, approximately 25% of all cases of preterm labour were caused by mother or foetus indications, 30% – due to premature rupture of membranes of foetus [8]. Many scholars argue that most of the premature labour occurs on the background of uncomplicated pregnancy [7, 9].

But, despite this, and summing up the results of our research, we can assume that a violation of microflora of vagina and skin of mammary glands is one of the causes of preterm labour. That is why women with violations of microflora of vagina and skin of mammary glands should be under the obstetrician supervision of obstetrician-gynaecologist. Each pregnant woman should be thoroughly examined to prevent complications during labour and avoid perinatal loss. In the detection of any pathology of female genital organs, it is necessary to conduct timely treatment.

Conclusions

Representatives of normal microflora: saprophytic Gram-positive and Gram-negative microorganisms, found on the skin of mammary glands of the control group of women, coincide with the data of the literature. In the pregnant women with preterm labour, there is the increase in the number of St. haemolyticus from 13% to 87%, the presence of representatives of pathogenic flora – St. aureus (in 20% of the examined).

In the study of microbiocenoses of mucous membrane of vagina in the examined women with threat of preterm labour compared with the control group, a sharp decrease in the number of lactic acid bacteria (Doderlein sticks, Lactobacillus) and the increase in the incidence of coccal flora (St. haemolyticus, St. aureus, Alpha-hemolytic streptococci, Beta-hemolytic streptococci).

Changes in the microbiocenosis of mammary glands and mucous membranes of vagina of pregnant women with preterm labour may indicate the presence of opportunistic microflora or personal hygiene or the presence of associated bacterial infections. Perhaps this is one of the causes of preterm birth in pregnant women.

The carriage of opportunistic and pathogenic coccalmicroflora can contribute to inflammatory purulent-septic processes in infants and new-borns.

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NANOTUBS INCREASE TETRACHLOROMETHANE INDUCED OXIDATIVE STRESS

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Background. *The unique physical and chemical properties of carbon nanotubes determine wide-ranging prospects for their use in biology and medicine. The capability of nanotubes to transport medicines and chemicals inside a cell makes the possibility of classical toxicants toxicity increase in case of their intake to the body with nanotubes, an urgent issue.*

Objective. *The aim of the research was to study the effect of carbon nanotubes on the capability of the chemical toxicant tetrachloromethane (TCM) to induce oxidative stress in serum and liver of rats.*

Methods. *The experiments were performed on outbred male rats, which were administered intraperitoneally with 0.5 ml of suspension of single-walled, multi-walled or multi-walled functionalized COOH nanotubes (60 mg/kg) only or together with TCM (2 ml/kg). The animals were taken out of the experiment in 3, 6 and 48 hours after the administration of the nanotubes and TCM. The activity of catalase, superoxide dismutase, the content of thiobarbituric acid reactive substances (TARS), reduced glutathione, ceruloplasmin and total antioxidant activity of serum were determined in serum and liver.*

Results. *It was established that under the influence of multi-walled carbon nanotubes the studied parameters changed significantly. The administration of tetrachloromethane to rats caused significant changes in all indicators. Maximal changes in the rates were recorded in the group of animals that were administered with carbon nanotubes and tetrachloromethane togeather. In this case, a number of the studied parameters of blood and liver significantly changed compare to the similar indicators of the group of animals, which were administered with the chemical toxicant only.*

Conclusions. *Carbon nanotubes increase the capability of the chemical toxicant tetrachloride to cause oxidative stress in liver and serum.*

KEY WORDS: **carbon nanotubes; tetrachloromethane; oxidative stress; rats.**

Introduction

Nanotechnology today is the most promising direction in the development of world science. Nanomaterials have caused a step forward in many industries and are used in our overall life. Carbon nanotubes (CNT) are one of the priority types of nanomaterials. They are multifunctional materials that are actively studied due to their unique physical and chemical properties [2, 6]. They exist in various forms and can be chemically modified by functional groups of biomolecules. CNT have unique mechanical, electrical and thermal properties and are widely used in various industries. Nanotubes are a promising nanomaterial for medical use due to their really high biocompatibility with blood, bones, cartilages and soft tissues [7, 9]. They can be used to create artificial heart valves, for the diagnosis and treatment of cancer, as well as for the transport of proteins, antigens, genes, vaccines and medicinal substances into a cell.

Due to everyday increase of nanomaterial use, less attention is paid to the possible negative effects of nanoparticles on environment and on people's health as a whole [14]. Small size, specific structure, large surface area, and chemical composition alert of possible toxic effects on the human body. Apart from the direct influence of carbon nanotubes on cells, they may interact with classical toxicants, e.g. tetrachloromethane (TCM). Currently, the issue of biological effects of nanoparticles in case of their intake to the body together with traditional toxicants is urgent. Thus it is necessary to study the toxicological properties of carbon nanotubes alone as well as in case of their intake to the body together with a toxicant.

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The aim of the research was to study the effect of carbon nanotubes on the capability of the chemical toxicant tetrachloromethane (TCM) to induce oxidative stress in serum and liver of experimental rats.

Methods

The experiments were performed on outbred male rats, 160 g in weight, which were kept on a standard vivarium diet. Single-walled (SWNT), multi-waledl (MWNT) and multi-walled functionalized (MWNT-COOH) nanotubes were administered to the animals in suspension (0.5 ml) intraperitoneally at a dose of 60 mg/kg. TCM was administered intraperitoneally in 50% oily solution at a dose of 2 ml/kg just the once. Dispersion of nanoparticles in distilled water or TCM solution was carried out by means of the ultrasonic disperser UZDN-M750T (20–25 kHz, 750 W) for 5 minutes. The experimental animals were divided into 8 groups: the 1st – the control (intact rats), administered with physical solution (0.5 ml/kg); the 2nd – the rats administered with SWNT, the 3rd – the animals administered with MWNT, the 4th – the rats administered with MWNT-COOH, the 5th – the animals administered with TCM, the 6th – the rats administered with SWNT suspension together with TCM, the 7th – the rats administered with thesuspension of MWNT+TCM, the 8th – the animals administered with the suspension of MWNT-COOH+TCM. The animals were taken out of the experiment under thiopental anesthesia in 3, 6 and 48 hours after the injection. Liver homogenate and blood serum were the objects of the study.

The animals were kept and the experiments were conducted in accordance with the guidelines of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

The state of antioxidant system was evaluated by the activity of enzymes of superoxide dismutase (SOD) [8], catalase (CT) [4], the content of ceruloplasmin (CP) [10] and reduced glutathione (GSH) [3]. The development of oxidative processes in the body was evidenced by the content of products that react with thiobarbituric acid (TBARs) [1]. The total antioxidant activity (TAA) of plasma was also determined [13].

The nanopowder of single-walled carbon nanotubes (SWCN, 90%, 1–2 nm), multi-walled nanotubes (MWCN, 99%, 13–18 nm) and carboxyfunctionalized nanotubes (MWCN-COOH, 95%, 30-50 nm)) produced by USResearch Nanomaterials, Inc. (USA) were used in the experiment. Tetrachloromethane produced by Makrokhim (Ukraine) was used as a model toxicant.

Statistical processing of the results was performed at the Department of System Statistical Study of I. Horbachevsky Ternopil State Medical University using the software package Statsoft STATISTICA. The obtained indexes were compared using the Mann-Whitney non-parametric test. The changes were statistically significant at $p<0.05$.

Results

In 3 and 6 hours after the administration of MWNT, the activity of SOD significantly decreased in serum and liver compared to the control. After the administration of MWNT-COOH, the changes in the SOD content in both tissues were significant only by the 6th hour of the experiment. At the same time, SWNT did not caused significant changes of this parameter. Another antioxidant defense enzyme that functions in blood and intercepts reactive oxygen intermediates is the CP. The content of CP in the blood of the animals administered with MWNT significantly exceeded the control indices in 1.3 times by the 6th hour of the experiment. After administration of nanotubes to the experimental animals, the processes of lipoperoxidation increased that was evidenced by the increase in the content of TBARs in serum and liver. Thus, in cases of MWNT administration, the TBARs content in serum was significantly higher in 1.3 and 1.4 times compare to the control group of animals, respectively by the 3rd and 6th hours of the experiment. In cases of SWNT and MWNT-COOH administration, the significant increase of this indicator was evidenced only by the 6th hour after injection.

A significant increase in CT activity was observed in cases of the administration of SWNT and MWNT-COOH by the 6th hour of the experiment (in 1.2 and 1.4 times respectively), as well as in 1.3 and 1.5 times by the 3rd and 6th hours after the administration of MWNT.

The reduced glutathione is one of the main antioxidants of non-enzymatic nature, its deficiency in tissues or blood causes significant oxidative stress [12]. As presented in Table 1, the administration of MWNT to animals caused a significant decrease in the content of reduced glutathione in 3 and 6 hours after injection, respectively in 1.3 and 1.5 times compared to the control, as well as in 1.4 times in 6 hours after the MWNT-COOH administration. The plasma TAA varied equally to the GSH. It should be noted that all the indices changed wavelike,

Table 1. The influence of carbon nanotubes on the indices of oxidative stress intensity in blood serum and liver of rats (M±m, n=8)

Index	Groups of animals								
	Intact	SWNT			MWNT			MWNT-COOH	
		Time after the administration (hours)							
		3	6	48	3	6	48	3	6	48
	Blood plasma									
TBARs, μmol/l	7.81 ±0.43	8.05 ±0.51	9.37* ±0.49	7.35 ±0.38	10.05* ±0.56	11.09* ±0.61	8.18 ±0.39	8.95 ±0.41	10.11* ±0.43	7.95 ±0.41
GSH, mmol/l	2.73 ±0.19	2.55 ±0.16	2.17 ±0.13	2.61 ±0.14	2.14* ±0.15	1.72* ±0.14	2.65 ±0.16	2.41 ±0.15	1.98* ±0.14	2.52 ±0.18
CT, MAb/l	0.67 ±0.04	0.79 ±0.05	0.81* ±0.04	0.63 ±0.06	0.89* ±0.03	1.00* ±0.07	0.78 ±0.03	0.82 ±0.04	0.93* ±0.06	0.60 ±0.04
CP, mg/l	238.4 ±9.20	247.9 ±9.85	257.3 ±8.01	245.7 ±10.04	261.7 ±10.65	285.5* ±11.45	251.3 ±8.95	258.1 ±8.40	269.6* ±10.25	255.4 ±8.83
TAA, %	61.49 ±4.10	60.72 ±3.52	58.05 ±2.70	61.1 ±4.26	53.31 ±3.02	45.57* ±2.98	55.13 ±4.05	57.45 ±3.80	50.23* ±2.75	59.01 ±5.12
SOD, units/ ml	8.33 ±0.54	7.62 ±0.60	7.02 ±0.48	8.16 ±0.53	6.65* ±0.51	6.30* ±0.43	8.41 ±0.60	7.08 ±0.64	6.47* ±0.53	7.99 ±0.52
	Liver									
TBARs, μmol/kg	62.53 ±2.04	64.11 ±1.98	72.06 ±1.64	59.32 ±2.12	69.33 ±1.94	87.54 ±2.42	64.02 ±1.85	65.84 ±1.78	81.23 ±1.66	59.83 ±2.03
SOD, units/g	0.60 ±0.02	0.62 ±0.03	0,53* ±0.02	0.59 ±0.05	0.51* ±0.03	0.43* ±0.05	0.61 ±0.04	0.52 ±0.03	0.49* ±0.04	0.64 ±0.05

Note: * – significant differences compared to the control ($p<0.05$).

but in 48 hours after the administration of nanoparticles were normal again.

Thus, it was proved that multi-walled functionalized COOH nanotubes had the most significant toxic effect.

The administration of TCM to the animals caused significant disorders of antioxidant system (Table 2). Above all, the content of TBARs in serum and liver increased significantly in all periods of the study. Significant changes of SOD activity were evidenced ($p<0.05$ in all cases) with the maximum decrease by the 6th hour of the experiment (in 1.7 times in serum and in 1.6 times in liver). Consecutively, CT activity and CP content, quite the opposite, were significantly increased in all periods of the study. The maximum increase of the catalase activity (in 1.9 times compare to the intact animals) was evidenced by the 6th hour of the experiment. The concentration of another important antioxidant – GSH, under the chemical toxicant influence, decreased in 1.5, 1.7 and 1.4 times compare to the control group of animals ($p<0.05$ in all cases). TAA decreased significantly in 1.4, 1.5 and 1.3 times in the corresponding study periods.

Table 2. The influence of tetrachloromethane on the indices of oxidative stress in blood serum and liver of rats (M±m, n=8)

Index	Groups of animals			
		CCl_4		
	Intact	Time after the administration (hours)		
		3	6	48
	Blood plasma			
TBARs, μmol/l	7.81±0.43	10.73*±0.48	12.91*±0.54	9.03±0.45
GSH, mmol/l	2.73±0.19	1.83*±0.14	1.65*±0.15	1.98*±0.15
CT, MAb/l	0.67±0.04	1.08*±0.06	1.27*±0.07	0.93*±0.03
CP, mg/l	238.4±9.20	291.8*±8.47	322.6*±9.02	283.1*±9.11
TAA, %	61.49±4.10	44.51*±2.41	41.29*±2.14	47.21*±2.95
SOD, units/ml	8.33±0.54	6.12*±0.38	5.11*±0.42	6.57*±0.39
	Liver			
TBARs, μmol/kg	62.53±2.54	79.85*±3.77	101.04*±3.25	84.49*±2.98
SOD, units/g	0.60±0.02	0.43*±0.03	0.38*±0.02	0.41*±0.04

Note: * – significant differences compared to the control ($p<0.05$).

The most significant changes in the functioning of antioxidant system were evidenced in the animals administered with total tetrahloromethane and carbon nanotubes (Table 3). In this group of animals, significant changes were evidenced in all the studied parameters compare to the intact animals in all periods of the study. It should be noted that the most of indices of the animals administered with the nanotubes+tetrachloromethane combined were significantly lower than those in the corresponding periods in the animals administered with tetrachloromethane and no nanotubes.

Table 3. The influence of combined administration of carbon nanotubes and tetrachloromethane on the indices of oxidative stress in blood serum and liver of rats (M±m, n=8)

Index	Intact	SWNT+CCl_4			MWNT +CCl_4			MWNT-COOH+CCl_4		
		Time after the administration (hours)								
		3	6	48	3	6	48	3	6	48
	Blood plasma									
TBARs, μmol/l	7.81 ±0.43	10.83* ±0.59	13.27* ±0.63	9.92* ±0.57	14.12*# ±0.61	16.43*# ±0.60	10.18* ±0.51	12.53*# ±0.57	14.83*# ±0.59	9.98* ±0.56
GSH, mmol/l	2.73 ±0.19	1.68* ±0.10	1.56* ±0.12	1.92* ±0.14	1.38*# ±0.12	1.16*# ±0.16	1.65* ±0.13	1.44* ±0.10	1.21*# ±0.14	1.73* ±0.16
CT, MAb/l	0.67 ±0.04	1.18* ±0.07	1.30* ±0.06	0.99* ±0.05	1.33*# ±0.06	1.58*# ±0.07	1.08* ±0.06	1.21* ±0.09	1.51* ±0.08	1.02* ±0.06
CP, mg/l	238.4 ±9.20	311.2*# ±7.02	326.9* ±9.61	308.7* ±7.82	321.8*# ±8.63	345.3*# ±9.25	298.1* ±8.08	319.7* ±9.40	331.6* ±8.25	289* ±8.22
TAA, %	61.49 ±4.10	42.31* ±2.62	37.91* ±3.18	44.77* ±2.91	38.31* ±2.65	30.44*# ±2.73	41.63* ±2.22	40.82* ±2.18	34.61*# ±2.09	43.65 ±3.58
SOD, units/ml	8.33 ±0.54	5.98* ±0.38	5.05* ±0.32	6.10* ±0.41	5.27* ±0.38	4.65* ±0.34	5.49* ±0.39	5.18* ±0.44	4.82* ±0.39	5.73* ±0.42
	liver									
TBARs, mol/kg	62.53 ±2.04	87.18* ±2.68	103.7* ±2.64	79.5* ±2.22	98.68* ±3.04	118.0*# ±2.40	88.66* ±2.13	91.02* ±2.78	106.4* ±2.51	78.3* ±2.13
SOD, units/g	0.60 ±0.02	0.43* ±0.04	0.34* ±0.02	0.48 ±0.04	0.36* ±0.03	0.28*# ±0.02	0.41* ±0.03	0.38* ±0.03	0.33* ±0.01	0.45* ±0.04

Notes: * – significant differences compared to the control ($p<0.05$).
– significant differences compared to the group of animals administered with tetrachloromethane ($p<0.05$).

Discussion

The study results brought us to the conclusion that the capability of the chemical toxicant tetrachloromethane to cause oxidative stress in serum and liver was significantly increased in case of its combined administration with carbon nanotubes. The effect of increased bioavailability of tetrachloromethane due to the capability of carbon nanotubes to absorb the toxin on its surface and to contribute to its transport to tissues and cells is the most likely explanation for the toxicity synergy of the investigated factors. According to the results of our research, as well as to the literature, nanotubes, especially MWNT, are able to induce the oxidative processes in tissues. It was established that the toxicity of nanotubes depended on their structure, size and surface area, as well as on the environment they are found in. The toxicity increased when the size of the particles decreased [2, 9].

Conclusions

Carbon nanotubes are able to activate the oxidative processes in the tissues of the body. The carbon nanotubes are placed in the following order by the degree of toxicity: MWNT>MWNT-COOH>SWNT.

Carbon nanotubes increase the capability of the chemical toxicant tetrachloride to cause oxidative stress in liver and serum.

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CHRONIC ENTEROCOLITIS COMBINED WITH STREPTOZOTOCIN-INDUCED DIABETES IN RATS: MECHANISM OF OXIDATIVE STRESS DEVELOPMENT

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Background. *Despite numerous studies on chronic inflammatory processes in colon, the complex problem of chronic enterocolitis (CEC) remains relevant.*

Objective. *The aim of the research is to evaluate the lipid peroxide oxidation and antioxidant defence system in rats suffering from chronic enterocolitis development with underlying streptozocine-induced diabetes mellitus.*

Methods. *The study involved 106 white non-linear male rats. Diabetes mellitus (DM) was modeled by a single intraperitoneal administration of streptozotocin to animals (Sigma Aldrich, USA, at a dose of 60 mg/kg of body weight). CEC was induced by a free access of animals to 1.0% solution of carrageenan in drinking water for 1 month.*

Results. *In the wall of small intestine of DM rats, lipid peroxide oxidation increases and the activity of enzyme link of antioxidant defence decreases reliably. The course of experimental CEC in rats is accompanied by the increase in free radical oxidation in the wall of small intestine and simultaneous increase of enzyme activity of antioxidant defence system, compared to the control.*

In cases of CEC with underlying DM in rats, the development of oxidative stress in the wall of small intestine is caused by the statistically significant increase in levels of diene conjugates and thiobarbituric acid reactive substances, ($p<0.01$) and the decrease in activity of SOD and catalase.

Conclusions. *The activation of free radical reactions is an important non-specific mechanism of inflammation development in tissues of small and large intestine in cases of diabetes mellitus.*

KEY WORDS: **chronic enterocolitis; diabetes mellitus; lipid peroxidation; antioxidant defence system; experiment.**

Introduction

A difficult social and economic situation, deterioration of living conditions, aggressiveness of the environment and other negative stress factors contribute to an increase in incidence of digestive diseases in the population. Digestive diseases are one of the most common among chronic diseases in developed countries. According to a number of studies, their share is 8–10% [1–5]. Chronic enterocolitis (CEC) is one of the diseases, which are based on the combination of elements of inflammation and dystrophy of mucous membrane with functional disorders in small and large intestines. This disease takes account of about 10% of the total chronic pathology of digestive system organs, and its prevalence is 5–12 cases per 1000 people [6]. Despite numerous studies on chronic inflammatory processes in colon, the complex problem of chronic enterocolitis remains relevant [7, 8]. For a long time this term defined a variety of pathological conditions of intestine, which was due to the lack of sufficiently clear notions about the nature of the disease. From the standpoint of modern concepts, chronic enterocolitis should be considered as a clinical and morphological phenomenon characterized by pain and dyspeptic syndromes typical for intestinal diseases with morphologically determined signs of epithelium dystrophy, the decrease in crypts depth and development of various severity lymphoplasmatic infiltration [9]. According to the official data, the incidence of CEC in the world is 50–230 cases per 100,000 people [10]. About

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15–20% of the world's population suffer from CEC. In the United States, over 20 million adults have symptoms of this disease. [11]. The annual increase in number of enterocolitis patients in the world is 5–20 cases per 100,000 people. Epidemiological calculations in the US proved that in white population, CEC is found in 3–5 times more often than in African Americans, while Jews are threatened in 3.5 times more than non-Jewish people. The disease occurs in all age groups, but the highest incidence is in 20–40-year-old individuals [12]. In the overall structure of gastroenterological pathology in America, CEC ranks first and accounts for 28% of all cases of treatment by gastroenterologists. About 12% of the patients seek medical advice from a general practitioner with complaints specific to CEC. CEC is diagnosed the most commonly in young people: 13.5% of people are aged 15-34, 13% of them are 35–44 years old and 9% are aged 45 and older. Therefore, the disease causes great economic harm to the society both the cost of medical care and by indirect factors, which include a compensation for temporary disability. [13]. However, it should be noted that in modern literature there is controversial information on CEC as an independent nosological form, and the presence of functional intestinal disease – an irritable bowel syndrome, is mentioned more often, while the changes in the intestinal mucosa are interpreted by the authors as morphofunctional ones, which causes the complexity of clinical and laboratory diagnostics and different understanding of CEC [14, 15].

From the pathogenetic point of view, the diseases related to the class of free radical pathology are widespread, starting with birth (bronchopulmonary dysplasia, retinopathy in preterm infants, enterocolitis, etc.). [16, 17]. The weakening of antioxidant defence and uncontrolled enhancement of lipid peroxidation processes is one of the important links in the pathogenesis of autonomic dysfunction, atopic dermatitis, dental pathology, diabetes mellitus, as well as pathology of gastrointestinal tract, enterocolitis as well [18, 19]. In this case peroxide lipid oxidation products, including malonic dialdehyde, which destabilize cellular membranes, reach high concentrations in blood and tissues [20, 21]. To date, a large amount of data has been accumulated proving the participation of free radical processes in the pathogenesis of CEC [16].

Therefore, the aim of our study was to evaluate the lipid peroxide oxidation and antioxidant defence system in rats suffering from chronic enterocolitis development with underlying streptozocine-induced diabetes mellitus.

Methods

The study involved 106 white non-linear male rats, which were kept on a standard vivarium diet of I. Horbachevsky Ternopil State Medical University. During the experiment the principles of the European Convention for the Protection of Animals used for Experimental and Other Scientific Purposes were followed. The experimental rats were divided into four groups: the 1st – control (intact animals), the 2nd – animals with diabetes mellitus, the 3rd – animals with chronic enterocolitis, the 4th – animals with diabetes mellitus and chronic enterocolitis. Diabetes mellitus (DM) was modeled by a single intraperitoneal administration of streptozotocin to the animals aged 2 months, (Sigma Aldrich, USA, at a dose of 60 mg/kg of body weight) [22]. Right before its injection, streptozotocin was dissolved in 0.1 molar citrate buffer (pH 4.5); the control group received the appropriate amount of citrate buffer. The study involved the animals with the glucose rate at least 10.8 mmol/L 2 weeks after streptozotocin administration. Chronic enterocolitis was induced by a free access of animals to 1.0% solution of carrageenan in drinking water for 1 month [23, 24]. Euthanasia of animals was performed by heart puncture under anaesthesia, in accordance with the requirements of the Animal Care Committee [25].

The state of lipid peroxide oxidation (LPO) was evaluated by the concentration of diene conjugates (DC) and thiobarbituric acid reactive substances (TBARS). The content of DC was determined by direct spectrophotometry, the principle of which is to isolate native fatty acids by extraction with a mixture of equal volumes of heptane and isopropanol, followed by measuring the optical density of heptane phase of lipid extract. Absorption at a wavelength of 232 nm evidences the content of DC [26]. To determine TBARS, we used the method of M. Mihara (1980), which consists in the formation of a coloured complex by the interaction of lipid peroxide oxidation products with thiobarbituric acid, by means of a standard set. The activity of antioxidant enzymes: catalase, was recorded simultaneously with the LPO processes [27], superoxide dismutase [28].

The obtained data were subjected to statistical processing [29, 30]. To verify the conformity of the data samples with the normal dis-

tribution law, the calculation by Shapiro-Wilk test was applied. Due to the lack of data matching to the normal distribution at the significance rate $p<0.05$, the median characteristics were estimated: median (Me), first and third quartiles (Q25-Q75). The level of statistical significance of sample differences was evaluated using non-parametric Mann-Whitney U test. Differences were considered statistically significant at the achieved rate of $p<0.05$.

Results

The increase of free radical oxidation processes in small intestine wall in the presence of the studied pathologies has been established ($p<0.05$). For instance, in cases of streptozotocin-induced diabetes, the content of DC increased statistically significantly by 66.93% and of TBARS by 71.22%, compared to the control values (Table 1). As the lipid peroxide oxidation increased, the activity of enzyme system of antioxidant defence system decreased, in particular, the activity of SOD decreased by 38.02% and that of catalase by 58.82%.

Experimental CEC was accompanied by the increase of lipid peroxide oxidation in the wall of small intestine (DC by 35.16% and TBARS by 27.65%, $p<0.01$) with simultaneous increase of enzyme activity of antioxidant defence system (SOD by 29.90% and catalase by 21.65%, $p<0.01$) compared to the control (Table 1).

The statistically significant activation of free radical oxidation processes in the rats with CEC combined with DM (the level of DC increased by 104.84% and of TBARS by 115.02% respectively, $p<0.01$) was evidenced. A significant decrease in activity of SOD by 131.09% and of catalase by 21.65% ($p<0.001$), compared to the control, was found.

Discussion

Thus, in cases of experimental diabetes, the prooxidant-antioxidant disbalance in the wall of small intestine was characterized by the development of oxidative stress. According to the literature, the increase in blood glucose levels with its auto-oxidation is one of the causes of intensification of free radical oxidation [31].

In current studies there are no reliable data on the effect of systematic consumption of carrageenan on the body of an adult, child, foetus and when this supplement comprises the diet of pregnant women. Studying this problem in the clinic is very problematic, so there is an urgent need of studying the influence of carrageenan on metabolic parameters in an experimental model.

The obtained data of experimental CEC prove that under the conditions of normal digestion due to acid hydrolysis, carrageenan splits into low and high molecular particles, which trigger free radical processes. Previous studies evidence the involvement of macrophages in the absorption of carrageenan with the formation of heterolysosomes, which leads to the implementation of harmful effects of lysosomal enzymes. On the other hand, macrophages are established to be the source of free radicals [32]. In order to ensure antioxidant equilibrium, an antioxidant defence system is activated simultaneously against the action of the external factor, proving the mobilization of protective and adaptive mechanisms associated with the excessive

Table 1. Rates of free radical oxidation in the wall of small intestine of the rats with chronic enterocolitis combined with streptozotocin-induced diabetes, Me (Q25-Q75)

Rate	Control	DM (group 2)	CEC (group 3)	DM+CEC (group 4)
DC, st.un./g of tissue	2.89 (2.50; 3.25)	4.83* (4.25; 5.29)	3.91* (3.71; 4.09)	5.92* (5.76; 6.15) $p_{1,2}<0.01$
TBARS, mmol/mg	3.33 (2.78; 3.80)	5.71* (5.53; 5.95)	4.26* (4.00; 4.57)	7.16* (6.98; 7.31)* $p_{1,2}<0.01$
SOD activity, st. un/mg of proteins	17.17 (16.28; 18.18)	10.64* (8.95; 12.02)	22.30* (21.43; 23.35)	7.43* (6.38; 8.08) $p_{1,2}<0.001$
Catalase activity, mmol·min/mg of proteins	41.64 (40.78; 42.55)	17.15* (16.16; 17.65)	50.66* (51.88; 54.55)	11.46* (10.85; 12.07) $p_{1,2}<0.001$

Notes: * – the difference between the control and experimental groups is statistically significant ($p<0.05$–0.001); p_1 – the reliability value between the groups 2 and 4; p_2 – the reliability value between the groups 3 and 4.

production of superoxide anion radical. Darren N. Seril et al. note that oxidative stress and free radical damage to mucous membranes of small and large intestines are one of the signs of peptic ulcer and, probably, one of the factors of carcinogenesis under these conditions [33].

The inflow of toxin (carrageenan) into the systemic circulation largely depends on the state of cytoplasmic membranes of organs and tissues that perform barrier functions, where the intestine is very important [34, 35]. One of the main pathogenetic factors regulating the permeability of membranes is the activation of free radical oxidation processes, which are crucial mechanism that provides the availability of lipid-protein complexes of membrane for phospholipase and proteases respectively [36]. This fact justifies the results obtained by us regarding the statistically significant activation of free radical oxidation processes in the rats with CEC combined with DM.

Reducing SOD activity may be due to the damaging effects of free radicals on the metalloprotein complex of enzyme containing copper, zinc or manganese. Low activity of catalase may be associated with the increase in concentration of hydrogen ions, which leads to the development of a proton form of an enzyme having an altered catalytic activity. It should be noted that in the experimental group 4 the rates of LPO were the highest and those of the antioxidant system were the lowest, compared to the other experimental groups. Therefore, the activation of free radical reactions is an important non-specific mechanism of inflammation development in tissues of small and large intestine in cases of diabetes mellitus.

Conclusions

In the wall of small intestine of streptozotocin-induced diabetic rats, lipid peroxide oxidation increases (DC content is higher than in the control by 66.93% and TBARS by 71.22%, respectively $p<0.001$) and the activity of enzyme link of antioxidant defence decreases reliably (SOD by 38.02% and catalase by 58.82%).

The course of experimental chronic enterocolitis in rats is accompanied by the increase in free radical oxidation in the wall of small intestine (DC by 35.16% and TBARS by 27.65%, $p<0.01$) with simultaneous increase of enzyme activity of antioxidant defence system (SOD by 29.90% and catalase by 21.65%, $p<0.01$), compared to the control.

In cases of chronic enterocolitis with underlying diabetes mellitus in rats, the development of oxidative stress in the wall of small intestine is associated with the statistically significant increase in levels of DC (by 104.84%) and TBARS (by 115.02%), $p<0.01$ and the decrease in activity of SOD (by 131.09%) and catalase (by 21.65%).

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AGE-RELATED MORPHOMETRIC CHARACTERISTICS OF REMODELING OF ARTERIAL BED OF HIND LIMBS IN WHITE RATS WITH EXPERIMENTAL HYPERCHOLESTEROLEMIA

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Background. *The human organism is constantly affected by metabolic risk factors. All of them for a long time are asymptomatic and often form in early childhood long before clinical manifestations. However, morphogenesis and morphofunctional features of age-related structural changes in blood vessels of different sites are poorly studied.*

Objective. *To evaluate the morphometric characteristics of arterial bed remodeling of hind limbs in white rats of PRA and RA with experimentally induced hypercholesterolemia.*

Methods. *The tissues biopsies from femoral, knee and shin areas were studied by means of histological and morphometric methods.*

Results. *Experimental hypercholesterolemia caused remodeling of vessels with increasing thickness of medial and endothelial layer of vessels in the investigated parts of the rats' body. In this case, the external diameter of vessels increased only in femoral area in the reproductive group of rats. In the group of pre-productive animals, the external diameter of the vessels studied was more or less unaffected. Thus, increased thickness of intima and media led to a decrease in arteries capacity. The depth of vascular lesions and the decrease in functional capacity of blood vessels were directly proportional to the duration of the experiment. In the group of reproductive rats, the changes in vascular bed were more evident.*

Conclusions. *Objective study of the processes allows providing a scientific basis for further research and understanding of the pathomorphism of vascular diseases in humans.*

KEY WORDS: **hypercholesterolemia; arteries; remodelling; morphometria.**

BIOMEDICAL SCIENCES

Introduction

Cardiovascular diseases take the first place in the population mortality rate of the most countries of the world and are characterized by the increase in their prevalence rates, including those at young able-bodied age [1, 2]. According to state statistics [3], in Ukraine over the past 10 years the prevalence of obesity among adolescents (15-17 years) per 1000 children population has increased in 2.5 times [3]. The human and animal organism is constantly affected by metabolic risk factors: insulin resistance, hyperinsulinemia, dyslipidemia, arterial hypertension, abdominal obesity, hyperuricemia are the most significant. All of them are asymptomatic for a long time and often form in early childhood long before clinical manifestations [4]. One of the first reactions to the toxic effects of metabolites are structural changes of vessels. However, morphogenesis and morphofunctional features of age-related structural changes in blood vessels of different sites are poorly studied. In the literature the established data is interpreted ambiguously. So, defining the morphometric rearrangement of arteries of femoral, knee and shin area of hind limbs in rats of pre-reproductive (PRA) and reproductive age (RA) with hypercholesterolemia (HH) is very important. So, the aim of the research was to evaluate the features of morphometric characteristics of remodeling of arterial bed of hind limbs in white rats of PRA and RA with experimentally induced hypercholesterolemia.

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Methods

Studies were performed by means of 64 white rats. The experimental group consisted of 48 animals with biochemically confirmed hypercholesterolemia. They were divided into

2 groups: the first – 24 rats, 2–3 months-old in age, weighing 150–170 grams; and the second – 24 rats aged 10–11 months, weighing 230–250 grams. The control group consisted of rats aged 2–3 and 10–11 months comprising 8 animals in each subgroup. Hypercholesterolemia was modeled by feeding cholesterol at a dose of 0.5 g/kg with warmed vegetable oil. To suppress thyroid function, Mercazolil was used at a dose of 10 mg/kg [5, 6]. This mixture was administered by means of an intragastric probe.

The level of cholesterolemia was determined by a biochemical method by means of a semiautomatic biochemical analyzer Humalyzer 2000 (Germany) using a standard set of reagents and instructions for their use, Human (Germany).

The biopsies of the tissues of femoral, knee and shin areas were fixed in a 10% neutral formalin solution and, according to a standard procedure, were sealed with paraffin. The dewaxed microtome sections were stained with hematoxylin and eosin, resorcin-fuchsin by Weigert and van Gieson, fuchsin Hart, iron hematoxylin by Heidenhain and alcian blue. During histological preparations for morphometry, the microscopes SEOSCAN, MBI-15 were used. The image from the microscopes was displayed on a computer monitor using the programs VISION Color CCD Camera and InterVideoWinDVR. To determine the size of tissue structures of vascular wall, the images of histological sections were analysed using the computer programs VideoTest-5.0, Kaare Image Base. The external diameter (ED) of arteries, the internal diameter (ID) of arteries, the thickness of medial (TM), the thickness of intima (TI), the resistance index of bandwidth capacity of arteries (RIBCA) were determined. The latter was calculated as the ratio of wall area of vessel to the area of its lumen in percent [7]. Laboratory rats were taken out from the experiment by bleeding after intraperitoneal injection of sodium thiopental at a dose of 50 mg/kg of body weight in 15, 30 and 45 days.

The rats were kept and all experiments were carried out in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Experiments on Animals adopted by the First National Congress on Bioethics (Kiev, 2001).), the Helsinki Declaration of the World Medical Association (2000), the Order of the Ministry of Health of Ukraine No. 281 from January 11, 2000.

Results

The level of cholesterolemia in the rats of pre-reproductive age with experimental hypercholesterolemia in 15 days was (2.15±0.03) mmol/l, and in the rats of reproductive age – (2.20±0.03) mmol/l. In 30 days the level of cholesterolemia in the PRA rats was (2.68±0.04) mmol/l and in the RA rats – (2.82±0.03) mmol/l. In 45 days the cholesterolemia level in the PRA rats was (2.83±0.05) mmol/l and in the RA rats – (3.03±0.03) mmol/l. In the intact rats group, the level of cholesterolemia was (1.80±0.04) mmol/l in the PRA animals and (1.37±0.04) mmol/l in the RA animals.

Analysis of the obtained morphometric indices in the animals of PRA and RA with experimental HH proved that they had a tendency to increase in comparison with the same parameters in the animals of intact group. In the rats of reproductive age, the structural changes are presented in Table 1.

Thus, the external diameter of femoral artery of the intact animals of reproductive age increased from (525.41±2.70) μm to (534.84±1.98) μm, which was 1.8% at $p<0.01$ in 45 days of the experiment. The internal diameter of femoral artery decreased by 4.65% in 30 days and by 6.32% in 45 days of the research ($p<0.001$). TTM of femoral artery increased from (100.04±0.69) μm to (114.44±0.76) μm, which was 14.39% in 45 days of the experiment ($p<0.001$).

The index of resistance to the femoral artery capacity gradually increased from (161.04±1.50) to (195.86±2.85)% in 30 days of the experiment and to (205.88±2,21)% in 45 days ($p<0.001$) and was higher by 21,62% and 27,84% respectively (Fig. 1).

The external diameter of popliteal artery and tibia artery in the rats of reproductive age was more or less unchanged till the end of the experiment. The internal diameter of popliteal artery decreased in 30 days of the experiment by 4.33% and in 45 days – by 5.55% ($p<0.01$). The TTM of popliteal artery increased from (81.29±0.37) μm to (83.35±0.74) μm in 15 days (2.6% ($p<0.05$)), to (89.11±1.12) μm in 30 days (9.6% ($p<0.001$)), and up to (90.69±0.94) μm (11.56% ($p<0.001$)) in 45 days of the experiment. Bandwidth resistance index of popliteal artery increased by 5.56% in 15 days ($p<0.01$), in 30 days – by 20.55% ($p<0.001$), and in 45 days – by 24.53% ($p<0.001$).

The internal diameter of artery of tibial area in the reproductive age animals in 15 days decreased by 2.75% ($p<0.05$), in 30 days – by

Table 1. Morphometric indices of arteries of hind limbs of the rats of reproductive age with experimental hypercholesterolemia

Time of the experiment	Area studied	Parameters		
		ED, μm	**ID, μm**	**TTM, μm**
Intact animals	Femoral artery	525.41±2.70	325.32±1.98	100.04±0.69
	Popliteal artery	416.69±1.50	254.12±0.97	81.29±0.37
	Tibia artery	139.17±0.70	83.18±0.37	28.00±0.19
Within 15 days	Femoral artery	533.38±1.70*	321.17±2.52	106.11±1.01***
	Popliteal artery	416.91±1.90	250.21±1.71	83.35±0.74*
	Tibia artery	139.45±1.21	80.90±1.06*	29.27±0.32**
Within 30 days	Femoral artery	533.15±2.75*	310.23±1.97***	111.46±1.02***
	Popliteal artery	419.65±2.26	241.44±2.13***	89.11±1.12***
	Tibia artery	140.26±1.32	79.71±1.06**	30.27±0.43***
Within 45 days	Femoral artery	534.84±1.98**	305.96±1.41***	114.44±0.76***
	Popliteal artery	419.95±2.37	238.57±1.43***	90.69±0.94***
	Tibia artery	140.73±1.37	79.57±1.13**	30.58±0.66***

Notes: * – p<0.05; ** – p<0.01; *** – p<0.001
ED – external diameter; **ID** – internal diameter; **TTM** – thickness of tunica media.

4.2% (p<0.001) and in 45 days of the study – by 4.4% (p<0.001). Statistical significance (p<0.01) of TTM arteries of tibial area increased in 15 days of the experiment, and in 45 days of the experiment it was by 9.2% higher (p<0.001). The ARI in tibial area increased from (179.93±0.85)% to (216.80±7.92)% in 45 days (p<0.001) and was higher by 20.49%.

The tunica intima (TI) in the rats of reproductive age (RA) in 15 days of the experiment was significant only in the knee area (+4.8% (p<0.05)). Morphometric indices of other regions during this period were unreliable. After 30 days, the TI of femoral artery in the reproductive animals increased by 7.78% (p<0.001), and in 45 days – by 8.83% (p<0.001). In the popliteal region the TI increased by 8.00% (in 30 days) and by 10.45% (p<0.001) in 45 days of the experiment. The values of TI of tibial area increased by 8.62% in 30 days and by 8.95% (p<0.001) in 45 days.

In the animals of pre-reproductive age (PRA), the morphometric parameters were similar, although somewhat reduced (Table 2). Thus, the external diameter of femoral artery was more or less unchanged during the study. The internal diameter of femoral artery decreased by 2% in 15 days (p<0.05), by 5.44% in 30 days and by 670% in 45 days of the study (p<0.001).

The parameters of TM of femoral artery increased in 30 days by 8.93% from (69.53±0.85) μm to (75.74±1.15) μm (p<0.001); In 45 days it increased by 11.11% to (77.26±0.74) μm (p<0.001). The IHSS of femoral artery gradually increased in 15 days by 6.4% from (164.23±2.58) to (174.72±2.61)% (p<0.01), in 30 days of the experiment – by 19.79% to

Table 2. Morphometric parameters of arteries of hind limbs of the pre-reproductive age rats with experimental hypercholesterolemia, (M±m)

Time of the experiment	Area studied	Parameters		
		ED, μm	**ID, μm**	**TTM, μm**
Intact animals	Femoral artery	361.66±2.18	222.60±1.10	69.53±0.85
	Popliteal artery	295.09±1.66	179.79±1.43	57.65±0.79
	Tibia artery	94.83±0.87	56.54±0.47	19.15±0.29
Within 15 days	Femoral artery	361.50±2.48	218.25±1.54*	71.62±0.81
	Popliteal artery	295.69±1.66	174.59±1.41*	60.55±1.41*
	Tibia artery	95.01±0.63	55.24±0.64	19.88±0.23
Within 30 days	Femoral artery	362.00±2.44	210.51±1.53***	75.74±1.15***
	Popliteal artery	296.03±1.75	170.02±1.32***	63.00±0.84***
	Tibia artery	95.28±0.96	54.21±0.53**	20.53±0.32**
Within 45 days	Femoral artery	362.10±2.25	207.70±1.57***	77.26±0.74***
	Popliteal artery	296.83±1.84	169.98±1.46***	63.43±0.68***
	Tibia artery	95.77±0.83	54.04±0.39***	20.87±0.29***

Notes: * – p<0.05; ** – p<0.01; *** – p<0.001.

(196.74±4.48)% (p <0.001) and in 45 days – by 24,67% to (204.76±3,10)% (p<0.001) (Fig. 2).

The external diameter of popliteal artery and tibial artery in the PRA rats, (and in the RA animals) didn't change just about till the end of the experiment. The internal diameter of popliteal artery decreased in 15 days by 2.9% (p<0.05), in 30 days – by 5.44%, in 45 days – by 5.46% (p<0.001). The TM of popliteal artery increased in 15 days by 5% (p<0.05), in 30 days – by 9.28% (p<0.001) and in 45 days of the experiment – by 10.02%. The RIBCA of popliteal artery increased by 10.33% in 15 days (p<0.01), in 30 days – by 19.95%, and in 45 days – by 20.81% (p<0.001). The internal diameter of artery of tibial area decreased in 15 days of the study by 2.30%, in 30 days – by 4.11% (p<0.01), and in 45 days – by 4.43% (p<0.001). The TM of artery of tibial region increased by 7.26% (p<0.01) in 30 days of the experiment, and in 45 days of the experiment it increased by 8.98% (p<0.001). The RIBCA of tibial region arteries increased in 45 days of the experiment from (181.82±3.45) to (214.43±3.33)% (p<0.001) and was by 17.93% higher than the control.

The thickness of intima of arteries in the PRA rats with experimental hypercholesterolemia increased a bit less than in the rats of RA. In 15 days of the experiment, the thickening of the intima was not reliable at all levels of the study. In 30 days, the TI of femoral artery increased by 6.90% (p<0.01), and in 45 days – by 7.63% (p<0.001); the TI of popliteal artery increased in 30 days by 7.69% (p<0.01) and in 45 days – by 9.61% (p<0.001); the increase of TI of tibial artery was 6.58% (p<0.01) in 30 days and 8.40% (p<0.001) in 45 days of the experiment.

Discussion

Arteries remodeling is initiated as an adaptive process in response to altered circulatory conditions or to the influence of any humoral agents. The arterial vessels are shaped throughout life: the walls of vessels thicken, their rigidity increases. The endothelium dysfunction and its increased permeability are indicators of arteries aging. These negative processes result in an enhanced synthesis of vasoconstrictor substances: angiotensin II and endothelin. At the same time, vasodilation development factors: nitric oxide, prostacyclin decrease, are manifested [8, 9]. In conditions of insufficient amount of nitric oxide, the endothelium of vessels cannot fulfil its inherent functions, and the decrease in the amount of NO in the vessels is the cause of atherosclerosis, other cardiovascular diseases and one of the first signs of aging of vascular bed. Intima diffusely thickens due to the aging of arteries that is caused by the accumulation of extracellular matrix proteins, smooth myocytes, collagen, glycosaminoglycans in it. These factors contribute to the enhanced adhesion of monocytes to the luminal surface of endothelium and are one of the stages of atherosclerotic plaque formation in arteries. There is evidence that vessel intima

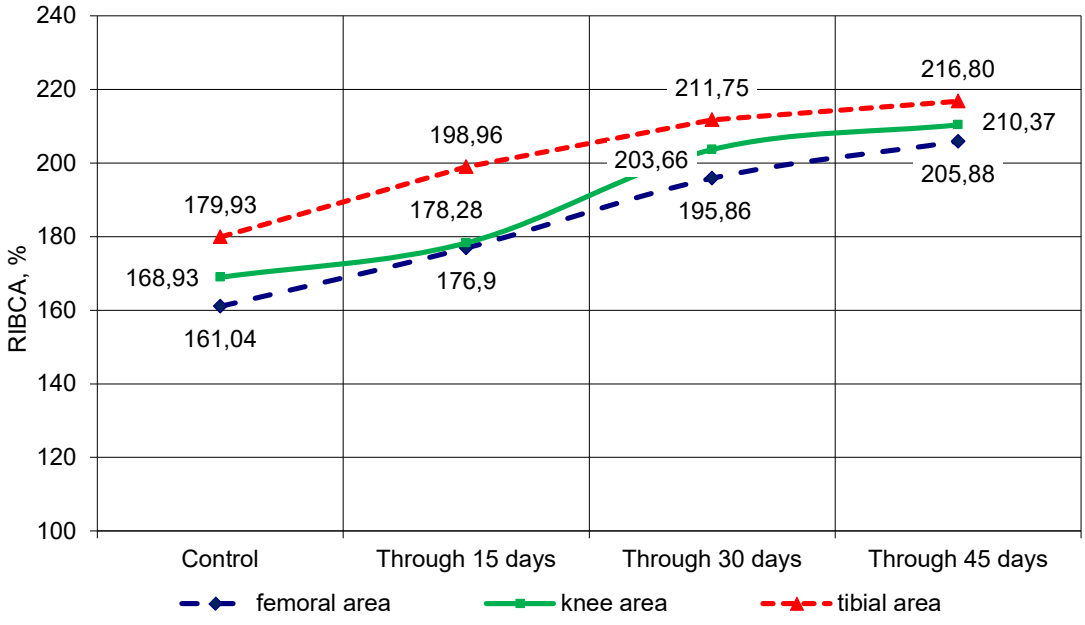

Fig. 1. The resistance index of the bandwidth capacity of arteries (RIBCA) of hind limbs in the rats of reproductive age with experimental hypercholesterolemia

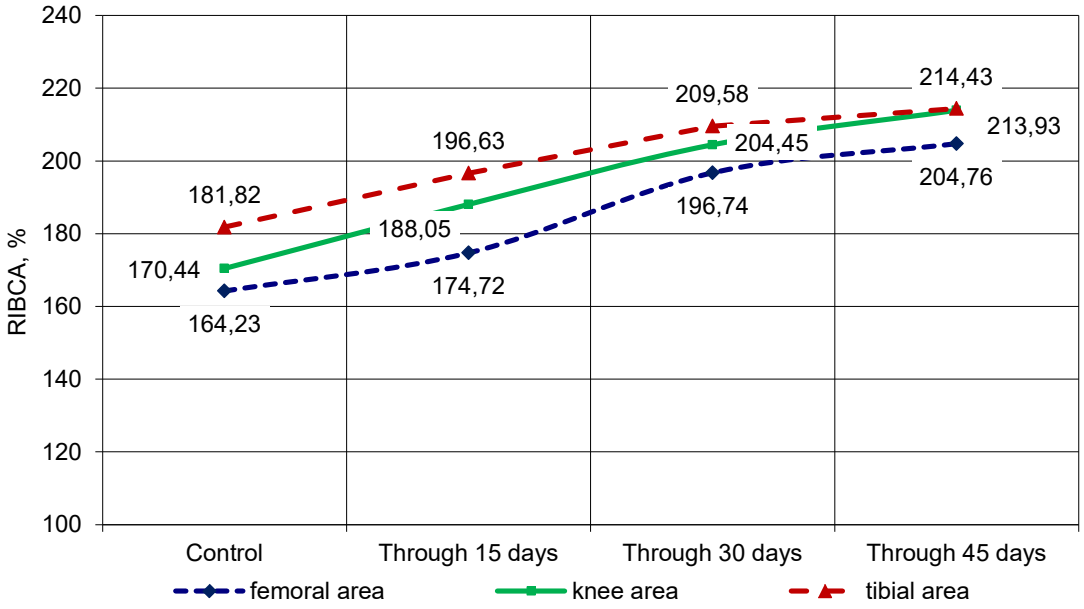

Fig. 2. The resistance index of bandwidth capacity of arteries (RIBCA) of hind limbs in the rats of pre-production age with experimental hypercholesterolemia

thickens in 2–3 times for the period of from 20 to 90 years old [10, 11].

The Proteins of extracellular matrix of the middle shell of arteries undergo significant structural and functional changes in aging. In the vessels there is an excessive deposition of calcium, transition of smooth myocytes from media into intima. Elastin of arteries is thinned that is characterized by fragmentation phenomena under the influence of matrix metalloproteinases and transforming growth factor-TGF-β [12, 13]. In contrast, the collagen protein synthesis is promoted with the assistance of angiotensin II, which increases the rigidity of collagen itself and vascular wall [14, 15]. Smooth myocytes in the intima of vessels are characterized by increased proliferation. This promotes the adhesion of blood cells to the endothelium of arterial vessels, thickening of intima, increasing its stiffness and development of atherosclerosis [16]. Morphometric indices in animals with hypercholesterolemia prove that hypercholesterolemia was accompanied by remodeling of predominantly femoral and popliteal arteries that was severe in the animals of reproductive age.

Conclusions

Experimental hypercholesterolemia in the rats of reproductive age was accompanied by remodeling of predominantly femoral and popliteal arteries. It should be noted that the intensity of these changes in the arteries of large caliber in the animals of pre-reproductive age was lower than in the animals of reproductive group, and remodeling of lower leg arteries was more significant in the animals of reproductive age.

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